

DEX-0109

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



AR

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : C12Q 1/68, C07K 14/47, C12N 5/10, C07K 16/00, G01N 33/574, A61K 38/17		A1	(11) International Publication Number: <b>WO 98/44159</b>
(21) International Application Number: <b>PCT/US98/06251</b>			(43) International Publication Date: 8 October 1998 (08.10.98)
(22) International Filing Date: 30 March 1998 (30.03.98)			
(30) Priority Data: 08/828,855	31 March 1997 (31.03.97)	US	
(71) Applicant: ABBOTT LABORATORIES [US/US]; 100 Abbott Park Road, Abbott Park, IL 60064-3500 (US).			(72) Inventors: BILLING-MEDEL, Patricia, A.; 1427 Sherwood Court, Gurnee, IL 60031 (US). COHEN, Maurice; 2026 Deerfield Road, Highland Park, IL 60035 (US). COLPITTS, Tracey, L.; 34365 North Circle Drive, Round Lake, IL 60073 (US). FRIEDMAN, Paula, N.; 462 Curnor Court, Deerfield, IL 60015 (US). GORDON, Julian; 307 East Sheridan Road, Lake Bluff, IL 60044 (US). GRANADOS, Edward, N.; 19 Montgomery Lane, Vernon Hills, IL 60061 (US). HAYDEN, Mark; 25059 N. Cedarwood Lane, Ingleside, IL 60041 (US). HODGES, Steven, C.; 169 Stonegate Road, Buffalo Grove, IL 60089 (US). KLASS, Michael, R.; 1606 Mulberry Drive, Libertyville, IL 60048 (US). KRATOCHVIL, Jon, D.; 7101 Fifth Avenue, Kenosha, WI 53143 (US). ROBERTS-RAPP, Lisa; 2090 Westfield Drive, Gurnee, IL 60031 (US). RUSSELL, John, C.; 8275 64th Court, Kenosha, WI 53142 (US). STROUPE, Stephen, D.; 945 Wilshire Drive, Libertyville, IL 60048 (US).
			(74) Agents: McCACKEN, Thomas, P. et al.; Robins & Associates, Suite 200, 90 Middlefield Road, Menlo Park, CA 94025 (US).
			(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
			<b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: REAGENTS AND METHODS USEFUL FOR DETECTING DISEASES OF THE GASTROINTESTINAL TRACT			
(57) Abstract			
A set of contiguous and partially overlapping cDNA sequences and polypeptides encoded thereby, designated as CS198 and transcribed from GI tract tissue, is described. These sequences are useful for detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining the predisposition of an individual to diseases and conditions of the GI tract, such as GI tract cancer. Also provided are antibodies which specifically bind to CS198-encoded polypeptide or protein, and agonists or inhibitors which prevent action of the tissue-specific CS198 polypeptide, which molecules are useful for the therapeutic treatment of GI tract diseases, tumors or metastases.			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

5

REAGENTS AND METHODS USEFUL FOR DETECTING  
DISEASES OF THE GASTROINTESTINAL TRACT

Background of the Invention

10        The invention relates generally to detecting diseases of the gastrointestinal tract organs, and more particularly, relates to reagents such as polynucleotide sequences and the polypeptide sequences encoded thereby, as well as methods which utilize these sequences, which are useful for detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining predisposition to 15        diseases and conditions of the GI tract such as cancer.

      The organs of the GI tract include the esophagus, stomach, small and large intestines, rectum and pancreas. Of the approximately 225,900 new cases of GI tract cancer projected for the United States during 1996, 131,200 will be due to colorectal cancer. Further, GI tract cancers will account for

20        approximately 127,070 related deaths (American Cancer Society statistics). In addition to its high incidence, GI tract cancers can be extremely lethal; for example, greater than 97% of pancreatic cancer patients will die of the disease. H.J. Wanebo, et al., Cancer 78:580-91 (1996).

25        Generally, the early detection of GI tract cancers at a pre-invasive stage dramatically reduces disease-related mortality. However, only few GI tract cancers are detected at this stage. For example, only 37% of colorectal cancers are detected at this stage by screening for premalignant polyps which can be removed before they progress to cancer. The primary methods used for colorectal cancer screening are fecal occult blood testing (FOBT) and flexible sigmoidoscopy. A. M. Cohen et al. In: Cancer: Principles and Practice of Oncology, Fourth Edition, pp. 929-977, Philadelphia, PA: J/B. Lippincott Co. (1993). Although FOBT is noninvasive, simple and inexpensive, its sensitivity is low; for example, sensitivity for detecting colorectal cancer was only 26% in one study. D.A. Ahlquist et al., JAMA 269: 1262-1267 (1993). Further, 30        35        although flexible sigmoidoscopy is highly sensitive for detecting early cancer and precursor polyps, it is invasive, costly, and too technically demanding to be

used for routine screening. D.F. Ransohoff, et al., JAMA 269: 1278-1281 (1993). In addition, only eight percent (8%) of pancreatic cancers and eighteen percent (18%) of stomach cancers are detected at a pre-invasive stage (American Cancer Society statistics). Thus, the need exists for improved screening 5 methods for detection of GI tract diseases such as cancer.

The standard procedures currently used for establishing a definitive diagnosis for a GI tract cancer include barium studies, endoscopy, biopsy and computed tomography (CT). These procedures are invasive and costly. Moreover, an erroneous diagnosis can result from any of these procedures due 10 to technical reasons, the subjective interpretation of results, or lack of sensitivity of the procedure. M. F. Brennan, et al. In: Cancer: Principles and Practice of Oncology, Fourth Edition, pp. 849-882, Philadelphia, PA: J.B. Lippincott Co. (1993).

After the diagnosis of a particular GI tract cancer is confirmed, staging is 15 performed to determine the anatomic extent of the disease. Staging is performed by a pathologist on tissue obtained by biopsy and/or surgery. Accurate staging is critical for predicting patient outcome and providing criteria for designing optimal therapy. Inaccurate staging can result in poor therapeutic decisions and is a major clinical problem in colorectal cancer. A need therefore exists for more 20 sensitive diagnostic procedures for staging GI tract cancers.

While surgical resection of the affected organ is typical therapy for a majority of patients diagnosed with GI tract cancers, some patients undergo radiation and/or chemotherapy. All of these patients need to be monitored in order to evaluate their response to therapy and to detect persistent or recurrent 25 disease and distant metastasis. A variety of markers including CEA and CA 19-9 can be assayed and the assay results used to monitor a patient's progress in conjunction with radiological procedures and colonoscopy. E.L. Jacobs, Curr. Probl. Cancer 15 (6):299-350 (1991). These monitoring techniques, however, have failed to provide an accurate and effective means to monitor the progress of 30 these patients.

Assays based upon the appearance of various disease markers in test samples such as blood, plasma or serum obtained by minimally invasive techniques, could provide low-cost and accurate information to aid the physician in diagnosing disease such as cancer, in selecting a therapy protocol, and in monitoring the success of the 35 chosen therapy. Such markers have been placed into several categories. The first category contains those markers which are elevated in disease. Examples include

detection of pancreatic, stomach, and esophageal cancers. Further, improved markers are needed to detect colorectal cancer.

It therefore would be advantageous to provide specific methods and reagents for detecting, diagnosing, staging, monitoring, prognosticating, 5 preventing or treating, or determining predisposition to diseases and conditions associated with the GI tract or to indicate possible predisposition to these conditions. Such methods would include assaying a test sample for products of a gene which are overexpressed in GI tract diseases and conditions such as cancer. Such methods may also include assaying a test sample for products of a 10 gene alteration associated with the GI tract disease or condition. Such methods may further include assaying a test sample for products of a gene whose distribution among the various tissues and compartments of the body have been altered by a GI tract-associated disease or condition such as cancer. Useful reagents include polynucleotide(s), or fragment(s) thereto which may be used in 15 diagnostic methods such as reverse transcriptase-polymerase chain reaction (RT-PCR), PCR, or hybridization assays of mRNA extracted from biopsied tissue, blood or other test samples; polypeptides or proteins which are the translation products of such mRNAs; or antibodies directed against these proteins. Drug treatment or gene therapy for diseases or conditions of the GI tract then can be 20 based on these identified gene sequences or their expressed proteins, and efficacy of any particular therapy can be monitored. Furthermore, it would be advantageous to have available alternative, non-surgical diagnostic methods capable of detecting early stage GI tract disease such as cancer.

25 Summary of the Invention

The present invention provides a method of detecting a target CS198 polynucleotide in a test sample which comprises contacting the test sample with at least one CS198-specific polynucleotide and detecting the presence of the target CS198 polynucleotide in the test sample. The CS198-specific polynucleotide has at 30 least 50% identity with a polynucleotide selected from the group consisting SEQUENCE ID NO 1, SEQUENCE ID NO 2, SEQUENCE ID NO 3, SEQUENCE ID NO 4, SEQUENCE ID NO 5, SEQUENCE ID NO 6, SEQUENCE ID NO 7, SEQUENCE ID NO 8, SEQUENCE ID NO 9, SEQUENCE ID NO 10, SEQUENCE ID NO 11, SEQUENCE ID NO 12, 35 SEQUENCE ID NO 13, SEQUENCE ID NO 14, SEQUENCE ID NO 15, SEQUENCE ID NO 16, SEQUENCE ID NO 17, SEQUENCE ID NO 18,

-3-

human chorionic gonadotropin (hCG) which is elevated in testicular cancer and trophoblastic disease, and alpha fetoprotein (AFP) which is elevated in hepatocellular carcinoma (HCC). E.L. Jacobs, supra. The second category includes qualitatively altered mRNA or protein markers in disease. Examples include mRNA 5 splice variants of CD 44 in bladder cancer and mutations in p53 protein in lung and colorectal cancer. Y. Matsumura et al. Journal of Pathology 175(Suppl): 108A (1995); W.P. Bennett, Cancer Detection and Prevention 19 (6): 503-511 (1995). The third category includes those protein markers which are normally expressed in a specific tissue, organ or organ system but which appear in an inappropriate body 10 compartment. For example, prostate specific antigen (PSA) is a normal protein which is secreted at high levels into the seminal fluid. PSA is present in very low levels in the blood of men with normal prostates but markedly elevated in the blood of patients with diseases of the prostate, including benign prostatic hyperplasia (BPH) and adenocarcinoma of the prostate. At high levels in the blood, PSA is a 15 strong indicator of prostate disease. P.H. Lange et al., Urology 33 (6 Suppl): 13 (1989). Similarly, carcinoembryonic antigen (CEA) is a normal component of the inner lining of the colon which is present in blood at low levels in people without colon disease. E. L. Jacobs, supra. However, the CEA concentration is markedly elevated in the blood, plasma or serum of many patients diagnosed with colon 20 disease including inflammatory bowel disease and adeno-carcinoma of the colon, and is used as an indicator of colorectal disease.

There are yet other examples of detecting disease markers in an inappropriate bodily compartment. In the case of metastatic cancer, the blood, bone marrow or lymph nodes may contain cells which have originated from the primary tumor and 25 which may express mRNA or protein markers representative of the primary tumor. For example, CEA and PSA have been demonstrated immunohistochemically in lymph nodes or bone marrow of patients with metastatic colorectal cancer and prostate cancer, respectively. B.R. Davidson, et al., Cancer 65:967-970 (1990); J.L. Mansi, et al., J. Urol., 139:545-548 (1988). In addition, RT-PCR has detected 30 CEA and PSA mRNAs at distant sites in patients with colon and prostate cancer, suggesting the presence of metastatic cells. M. Gerhard, et al., J. Clin. Oncol. 12:725-729 (1994); A.E. Katz, et al., Urology 43:765-775 (1994). Other compartments in which the inappropriate appearance of normal gene products may 35 be indicative of disease include but are not limited to, whole blood, urine, saliva, and stool. Currently, no universally acceptable marker(s) exist(s) for the early

further, the detectable label can be attached to a solid phase. Test kits useful for detecting target CS198 polynucleotides in a test sample are also provided which comprise a container containing at least one CS198-specific polynucleotide selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or 5 complements thereof. These test kits further comprise containers with tools useful for collecting test samples (such as, for example, blood, urine, saliva and stool). Such tools include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; and cups for collecting and stabilizing urine or stool samples. Collection materials, such as papers, cloths, 10 swabs, cups, and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. The collection materials also may be treated with or contain preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens.

The present invention also provides a purified polynucleotide or fragment 15 thereof derived from a CS198 gene. The purified polynucleotide is capable of selectively hybridizing to the nucleic acid of the CS198 gene, or a complement thereof. The polynucleotide has at least 50% identity with a polynucleotide selected from the group consisting of (a) SEQUENCE ID NOS 7-13 and complements thereof, (b) SEQUENCE ID NOS 15-26 and complements thereof, and (c) 20 fragments of SEQUENCE ID NOS 7-26. Further, the purified polynucleotide can be produced by recombinant and/or synthetic techniques. The purified recombinant polynucleotide can be contained within a recombinant vector. The invention further comprises a host cell transfected with the recombinant vector.

The present invention further provides a recombinant expression system 25 comprising a nucleic acid sequence that includes an open reading frame derived from CS198. The nucleic acid sequence has at least 50% identity with a sequence selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof. The nucleic acid sequence is operably linked to a control sequence compatible with a desired host. Also provided is a cell transfected with 30 this recombinant expression system.

The present invention also provides a polypeptide encoded by CS198. The polypeptide can be produced by recombinant technology, provided in purified form, or produced by synthetic techniques. The polypeptide comprises an amino acid sequence which has at least 50% identity with an amino acid sequence selected from 35 the group consisting of (a) SEQUENCE ID NOS 43-47, and (b) fragments of SEQUENCE ID NOS 42-47.

-5-

SEQUENCE ID NO 19, SEQUENCE ID NO 20, SEQUENCE ID NO 21,  
SEQUENCE ID NO 22, SEQUENCE ID NO 23, SEQUENCE ID NO 24,  
SEQUENCE ID NO 25, SEQUENCE ID NO 26, SEQUENCE ID NO 27  
("SEQUENCE ID NOS 1-27"), and fragments or complements thereof. Also, the  
5 CS198-specific polynucleotide may be attached to a solid phase prior to performing  
the method.

The present invention also provides a method for detecting CS198 mRNA in a test sample, which comprises performing reverse transcription (RT) with at least one primer in order to produce cDNA, amplifying the cDNA so obtained using  
10 CS198 oligonucleotides as sense and antisense primers to obtain CS198 amplicon, and detecting the presence of the CS198 amplicon as an indication of the presence of CS198 mRNA in the test sample, wherein the CS198 oligonucleotides have at least 50% identity to a sequence selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof. Amplification can be performed  
15 by the polymerase chain reaction. Also, the test sample can be reacted with a solid phase prior to performing the method, prior to amplification or prior to detection. This reaction can be a direct or an indirect reaction. Further, the detection step can comprise utilizing a detectable label capable of generating a measurable signal. The detectable label can be attached to a solid phase.

20 The present invention further provides a method of detecting a target CS198 polynucleotide in a test sample suspected of containing target CS198 polynucleotides, which comprises (a) contacting the test sample with at least one CS198 oligonucleotide as a sense primer and at least one CS198 oligonucleotide as an anti-sense primer, and amplifying same to obtain a first stage reaction product; (b)  
25 contacting the first stage reaction product with at least one other CS198 oligonucleotide to obtain a second stage reaction product, with the proviso that the other CS198 oligonucleotide is located 3' to the CS198 oligonucleotides utilized in step (a) and is complementary to the first stage reaction product; and (c) detecting the second stage reaction product as an indication of the presence of a target CS198 polynucleotide in the test sample. The CS198 oligonucleotides selected as reagents in the method have at least 50% identity to a sequence selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof. Amplification may be performed by the polymerase chain reaction. The test sample can be reacted either directly or indirectly with a solid phase prior to performing the  
30 method, or prior to amplification, or prior to detection. The detection step also comprises utilizing a detectable label capable of generating a measurable signal;  
35

Also provided is an antibody which specifically binds to at least one CS198 epitope. The antibody can be a polyclonal or monoclonal antibody. The epitope is derived from an amino acid sequence selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof. Assay kits for determining the 5 presence of CS198 antigen or anti-CS198 antibody in a test sample are also included. In one embodiment, the assay kits comprise a container containing at least one CS198 polypeptide having at least 50% identity with an amino acid sequence selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof. Further, the test kit can comprise a container with tools useful for collecting 10 test samples (such as blood, urine, saliva, and stool). Such tools include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; and cups for collecting and stabilizing urine or stool samples. Collection materials, such as papers, cloths, swabs, cups, and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. 15 These collection materials also may be treated with or contain preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens. Also, the polypeptide can be attached to a solid phase.

Another assay kit for determining the presence of CS198 antigen or anti-CS198 antibody in a test sample comprises a container containing an antibody which 20 specifically binds to a CS198 antigen, wherein the CS198 antigen comprises at least one CS198-encoded epitope. The CS198 antigen has at least about 60% sequence similarity to a sequence of a CS198-encoded antigen selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof. These test kits can further comprise containers with tools useful for collecting test samples (such as 25 blood, urine, saliva, and stool). Such tools include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; cups for collecting and stabilizing urine or stool samples. Collection materials, such as papers, cloths, swabs, cups and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. These collection materials also 30 may be treated with, or contain, preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens. The antibody can be attached to a solid phase.

A method for producing a polypeptide which contains at least one epitope of CS198 is provided, which method comprises incubating host cells transfected with 35 an expression vector. This vector comprises a polynucleotide sequence encoding a polypeptide, wherein the polypeptide comprises an amino acid sequence having at

least 50% identity with a CS198 amino acid sequence selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof.

A method for detecting CS198 antigen in a test sample suspected of containing CS198 antigen also is provided. The method comprises contacting the test sample with an antibody or fragment thereof which specifically binds to at least one epitope of CS198 antigen, for a time and under conditions sufficient for the formation of antibody/antigen complexes; and detecting the presence of such complexes containing the antibody as an indication of the presence of CS198 antigen in the test sample. The antibody can be attached to a solid phase and may be either a monoclonal or polyclonal antibody. Furthermore, the antibody specifically binds to at least one CS198 antigen selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof.

Another method is provided which detects antibodies which specifically bind to CS198 antigen in a test sample suspected of containing these antibodies. The method comprises contacting the test sample with a polypeptide which contains at least one CS198 epitope, wherein the CS198 epitope comprises an amino acid sequence having at least 50% identity with an amino acid sequence encoded by a CS198 polynucleotide, or a fragment thereof. Contacting is carried out for a time and under conditions sufficient to allow antigen/antibody complexes to form. The method further entails detecting complexes which contain the polypeptide. The polypeptide can be attached to a solid phase. Further, the polypeptide can be a recombinant protein or a synthetic peptide having at least 50% identity with an amino acid sequence selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof.

The present invention provides a cell transfected with a CS198 nucleic acid sequence that encodes at least one epitope of a CS198 antigen, or fragment thereof. The nucleic acid sequence is selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof.

A method for producing antibodies to CS198 antigen also is provided, which method comprises administering to an individual an isolated immunogenic polypeptide or fragment thereof, wherein the isolated immunogenic polypeptide comprises at least one CS198 epitope. The immunogenic polypeptide is administered to the individual in an amount sufficient to produce an immune response. The isolated, immunogenic polypeptide comprises an amino acid sequence selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof.

Another method for producing antibodies which specifically bind to CS198 antigen is disclosed, which method comprises administering to a mammal a plasmid comprising a nucleic acid sequence which encodes at least one CS198 epitope derived from an amino acid sequence selected from the group consisting of

5 SEQUENCE ID NOS 42-47, and fragments thereof. The plasmid is administered in an amount such that the plasmid is taken up by cells in the individual and expressed at levels sufficient to produce an immune response.

Also provided is a composition of matter that comprises a CS198 polynucleotide of at least about 10-12 nucleotides having at least 50% identity with a

10 polynucleotide selected from the group consisting of (a) SEQUENCE ID NOS 7-13 and complements thereof, (b) SEQUENCE ID NOS 15-26 and complements thereof, and (c) fragments of SEQUENCE ID NOS 7-26. The CS198 polynucleotide encodes an amino acid sequence having at least one CS198 epitope.

Another composition of matter provided by the present invention comprises a

15 polypeptide with at least one CS198 epitope of about 8-10 amino acids. The polypeptide comprises an amino acid sequence having at least 50% identity with an amino acid sequence selected from the group consisting of (a) SEQUENCE ID NOS 43-47, and (b) fragments of SEQUENCE ID NOS 42-47. Also provided is a gene, or a fragment thereof, coding for a CS198 polypeptide having at least 50% identity

20 with SEQUENCE ID NO 47; and a gene, or a fragment thereof, comprising DNA having at least 50% identity with SEQUENCE ID NO 26.

#### Brief Description of the Drawings

Figures 1A-1G show the nucleotide alignment of exon sequences derived

25 from genomic clone g2804590 (SEQUENCE ID NOS 1-6), clones 2682428 (SEQUENCE ID NO 7), 2682469 (SEQUENCE ID NO 8), 3359342 (SEQUENCE ID NO 9), 1736231 (SEQUENCE ID NO 10), 1734520 (SEQUENCE ID NO 11), 2596108 (SEQUENCE ID NO 12), 3388863 (SEQUENCE ID NO 13), g2322685 (SEQUENCE ID NO 14), 3988413 (SEQUENCE ID NO 15), 3615515

30 (SEQUENCE ID NO 16), 2055371 (SEQUENCE ID NO 17), 1431231 (SEQUENCE ID NO 18), 3253860 (SEQUENCE ID NO 19), 1753756 (SEQUENCE ID NO 20), 1887713 (SEQUENCE ID NO 21), 1803052 (SEQUENCE ID NO 22), 889029 (SEQUENCE ID NO 23), 2620906 (SEQUENCE ID NO 24), 1754901 (SEQUENCE ID NO 25); the full-length

35 sequence of clone 2055371 (designated as clone 2055371IH (SEQUENCE ID NO 26)); and the consensus sequence (SEQUENCE ID NO 27) derived therefrom.

Figure 2 shows the contig map depicting the formation of the consensus nucleotide sequence (SEQUENCE ID NO 27) from the nucleotide alignment of exon sequences derived from genomic clone g2804590 (SEQUENCE ID NOS 1-6) and overlapping clones 2682428 (SEQUENCE ID NO 7), 2682469 (SEQUENCE ID NO 8), 3359342 (SEQUENCE ID NO 9), 1736231 (SEQUENCE ID NO 10), 1734520 (SEQUENCE ID NO 11), 2596108 (SEQUENCE ID NO 12), 3388863 (SEQUENCE ID NO 13), g2322685 (SEQUENCE ID NO 14), 3988413 (SEQUENCE ID NO 15), 3615515 (SEQUENCE ID NO 16), 2055371 (SEQUENCE ID NO 17), 1431231 (SEQUENCE ID NO 18), 3253860 (SEQUENCE ID NO 19), 1753756 (SEQUENCE ID NO 20), 1887713 (SEQUENCE ID NO 21), 1803052 (SEQUENCE ID NO 22), 889029 (SEQUENCE ID NO 23), 2620906 (SEQUENCE ID NO 24), 1754901 (SEQUENCE ID NO 25), and 2055371IH (SEQUENCE ID NO 26).

Figure 3 is a scan of an ethidium bromide stained agarose gel of RNA from various tissues and the corresponding Northern blot of RNA using a CS198 radiolabeled probe.

Figure 4 and Figure 5 are scans of stained agarose gels of CS198-specific primed PCR amplification products.

Figure 6 is a scan of a Western blot of a panel of human tissue extracts using antiserum against a CS198 synthetic peptide. The arrow indicates the band representing antibodies which bind to the 45 kD protein.

#### Detailed Description of the Invention

The present invention provides a gene, or a fragment thereof, which codes for a CS198 polypeptide having at least about 50% identity with SEQUENCE ID NO 47. The present invention further encompasses a CS198 gene, or a fragment thereof, comprising DNA which has at least about 50% identity with SEQUENCE ID NO.

The present invention also provides methods for assaying a test sample for products of a gastrointestinal tract (GI tract) tissue gene designated as CS198, which comprises making cDNA from mRNA in the test sample, and detecting the cDNA as an indication of the presence of GI tract tissue gene CS198. The method may include an amplification step, wherein one or more portions of the mRNA from CS198 corresponding to the gene or fragments thereof, is amplified. Methods also are provided for assaying for the translation products of CS198. Test samples which may be assayed by the methods provided herein include tissues, cells, body

-11-

fluids and secretions. The present invention also provides reagents such as oligonucleotide primers and polypeptides which are useful in performing these methods.

Portions of the nucleic acid sequences disclosed herein are useful as primers 5 for the reverse transcription of RNA or for the amplification of cDNA; or as probes to determine the presence of certain mRNA sequences in test samples. Also disclosed are nucleic acid sequences which permit the production of encoded polypeptide sequences which are useful as standards or reagents in diagnostic immunoassays, as targets for pharmaceutical screening assays and/or as components 10 or as target sites for various therapies. Monoclonal and polyclonal antibodies directed against at least one epitope contained within these polypeptide sequences are useful as delivery agents for therapeutic agents as well as for diagnostic tests and for screening for diseases or conditions associated with CS198, especially GI tract cancer. Isolation of sequences of other portions of the gene of interest can be 15 accomplished utilizing probes or PCR primers derived from these nucleic acid sequences. This allows additional probes of the mRNA or cDNA of interest to be established, as well as corresponding encoded polypeptide sequences. These additional molecules are useful in detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining the predisposition to diseases 20 and conditions of the GI tract, such as GI tract cancer, characterized by CS198, as disclosed herein.

Techniques for determining amino acid sequence "similarity" are well-known in the art. In general, "similarity" means the exact amino acid to amino acid comparison of two or more polypeptides at the appropriate place, where amino acids 25 are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" then can be determined between the compared polypeptide sequences. Techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining the nucleotide sequence of the mRNA for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded thereby, and comparing this to a second amino acid sequence. In general, "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two 30 polynucleotides or polypeptide sequences, respectively. Two or more polynucleotide sequences can be compared by determining their "percent identity." 35 Two or more amino acid sequences likewise can be compared by determining their "percent identity." The programs available in the Wisconsin Sequence Analysis

-12-

Package, Version 8 (available from Genetics Computer Group, Madison, WI), for example, the GAP program, are capable of calculating both the identity between two polynucleotides and the identity and similarity between two polypeptide sequences, respectively. Other programs for calculating identity or similarity between 5 sequences are known in the art.

The compositions and methods described herein will enable the identification of certain markers as indicative of a GI tract tissue disease or condition; the information obtained therefrom will aid in the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining diseases or 10 conditions associated with CS198, especially GI tract cancer. Test methods include, for example, probe assays which utilize the sequence(s) provided herein and which also may utilize nucleic acid amplification methods such as the polymerase chain reaction (PCR), the ligase chain reaction (LCR), and hybridization. In addition, the nucleotide sequences provided herein contain open reading frames from which an 15 immunogenic epitope may be found. This epitope is believed to be unique to the disease state or condition associated with CS198. It also is thought that the polynucleotides or polypeptides and protein encoded by the CS198 gene are useful as a marker. This marker is either elevated in disease such as GI tract cancer, altered in disease such as GI tract cancer, or present as a normal protein but appearing in an 20 inappropriate body compartment. The uniqueness of the epitope may be determined by (i) its immunological reactivity and specificity with antibodies directed against proteins and polypeptides encoded by the CS198 gene, and (ii) its nonreactivity with any other tissue markers. Methods for determining immunological reactivity are well-known and include, but are not limited to, for example, radioimmunoassay 25 (RIA), enzyme-linked immunoabsorbent assay (ELISA), hemagglutination (HA), fluorescence polarization immunoassay (FPIA), chemiluminescent immunoassay (CLIA) and others. Several examples of suitable methods are described herein.

Unless otherwise stated, the following terms shall have the following meanings:

30 A polynucleotide "derived from" or "specific for" a designated sequence refers to a polynucleotide sequence which comprises a contiguous sequence of approximately at least about 6 nucleotides, preferably at least about 8 nucleotides, more preferably at least about 10-12 nucleotides, and even more preferably at least about 15-20 nucleotides corresponding, i.e., identical or complementary to, a region 35 of the designated nucleotide sequence. The sequence may be complementary or identical to a sequence which is unique to a particular polynucleotide sequence as

-13-

determined by techniques known in the art. Comparisons to sequences in databanks, for example, can be used as a method to determine the uniqueness of a designated sequence. Regions from which sequences may be derived, include but are not limited to, regions encoding specific epitopes, as well as non-translated 5 and/or non-transcribed regions.

The derived polynucleotide will not necessarily be derived physically from the nucleotide sequence of interest under study, but may be generated in any manner, including, but not limited to, chemical synthesis, replication, reverse transcription or transcription, which is based on the information provided by the 10 sequence of bases in the region(s) from which the polynucleotide is derived. As such, it may represent either a sense or an antisense orientation of the original polynucleotide. In addition, combinations of regions corresponding to that of the designated sequence may be modified in ways known in the art to be consistent with the intended use.

15 A "fragment" of a specified polynucleotide refers to a polynucleotide sequence which comprises a contiguous sequence of approximately at least about 6 nucleotides, preferably at least about 8 nucleotides, more preferably at least about 10-12 nucleotides, and even more preferably at least about 15-20 nucleotides corresponding, i.e., identical or complementary to, a region of the specified 20 nucleotide sequence.

25 The term "primer" denotes a specific oligonucleotide sequence which is complementary to a target nucleotide sequence and used to hybridize to the target nucleotide sequence. A primer serves as an initiation point for nucleotide polymerization catalyzed by either DNA polymerase, RNA polymerase or reverse transcriptase.

The term "probe" denotes a defined nucleic acid segment (or nucleotide analog segment, e.g., PNA as defined hereinbelow) which can be used to identify a specific polynucleotide present in samples bearing the complementary sequence.

30 "Encoded by" refers to a nucleic acid sequence which codes for a polypeptide sequence, wherein the polypeptide sequence or a portion thereof contains an amino acid sequence of at least 3 to 5 amino acids, more preferably at least 8 to 10 amino acids, and even more preferably at least 15 to 20 amino acids from a polypeptide encoded by the nucleic acid sequence. Also encompassed are polypeptide sequences which are immunologically identifiable with a polypeptide 35 encoded by the sequence. Thus, a "polypeptide," "protein," or "amino acid" sequence has at least about 50% identity, preferably about 60% identity, more

preferably at least about 75% similarity, more preferably about 85% similarity, and  
5 most preferably about 95% or more similarity to a polypeptide or amino acid  
sequence of CS198. This amino acid sequence can be selected from the group  
consisting of SEQUENCE ID NO 42, SEQUENCE ID NO 43, SEQUENCE ID NO  
44, SEQUENCE ID NO 45, SEQUENCE ID NO 46, and fragments thereof.

A "recombinant polypeptide," "recombinant protein," or "a polypeptide  
10 produced by recombinant techniques," which terms may be used interchangeably  
herein, describes a polypeptide which by virtue of its origin or manipulation is not  
associated with all or a portion of the polypeptide with which it is associated in  
nature and/or is linked to a polypeptide other than that to which it is linked in nature.  
A recombinant or encoded polypeptide or protein is not necessarily translated from a  
15 designated nucleic acid sequence. It also may be generated in any manner, including  
chemical synthesis or expression of a recombinant expression system.

The term "synthetic peptide" as used herein means a polymeric form of  
amino acids of any length, which may be chemically synthesized by methods well-  
known to the routineer. These synthetic peptides are useful in various applications.

20 The term "polynucleotide" as used herein means a polymeric form of  
nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term  
refers only to the primary structure of the molecule. Thus, the term includes double-  
and single-stranded DNA, as well as double- and single-stranded RNA. It also  
includes modifications, such as methylation or capping and unmodified forms of the  
25 polynucleotide. The terms "polynucleotide," "oligomer," "oligonucleotide," and  
"oligo" are used interchangeably herein.

30 "A sequence corresponding to a cDNA" means that the sequence contains a  
polynucleotide sequence that is identical or complementary to a sequence in the  
designated DNA. The degree (or "percent") of identity or complementarity to the  
cDNA will be approximately 50% or greater, preferably at least about 70% or  
greater, and more preferably at least about 90% or greater. The sequence that  
corresponds to the identified cDNA will be at least about 50 nucleotides in length,  
preferably at least about 60 nucleotides in length, and more preferably at least about  
35 70 nucleotides in length. The correspondence between the gene or gene fragment of  
interest and the cDNA can be determined by methods known in the art and include,  
for example, a direct comparison of the sequenced material with the cDNAs

-15-

described, or hybridization and digestion with single strand nucleases, followed by size determination of the digested fragments.

“Purified polynucleotide” refers to a polynucleotide of interest or fragment thereof which is essentially free, e.g., contains less than about 50%, preferably less than about 70%, and more preferably less than about 90%, of the protein with which the polynucleotide is naturally associated. Techniques for purifying polynucleotides of interest are well-known in the art and include, for example, disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide(s) and proteins by ion-exchange chromatography, affinity chromatography and sedimentation according to density.

“Purified polypeptide” or “purified protein” means a polypeptide of interest or fragment thereof which is essentially free of, e.g., contains less than about 50%, preferably less than about 70%, and more preferably less than about 90%, cellular components with which the polypeptide of interest is naturally associated. Methods for purifying polypeptides of interest are known in the art.

The term “isolated” means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or DNA or polypeptide, which is separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotide could be part of a vector and/or such polynucleotide or polypeptide could be part of a composition, and still be isolated in that the vector or composition is not part of its natural environment.

“Polypeptide” and “protein” are used interchangeably herein and indicate at least one molecular chain of amino acids linked through covalent and/or non-covalent bonds. The terms do not refer to a specific length of the product. Thus peptides, oligopeptides and proteins are included within the definition of polypeptide. The terms include post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. In addition, protein fragments, analogs, mutated or variant proteins, fusion proteins and the like are included within the meaning of polypeptide.

A “fragment” of a specified polypeptide refers to an amino acid sequence which comprises at least about 3-5 amino acids, more preferably at least about 8-10 amino acids, and even more preferably at least about 15-20 amino acids derived from the specified polypeptide.

“Recombinant host cells,” “host cells,” “cells,” “cell lines,” “cell cultures,” and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities refer to cells which can be, or have been, used as recipients for recombinant vector or other transferred DNA, and include the original 5 progeny of the original cell which has been transfected.

As used herein “replicon” means any genetic element, such as a plasmid, a chromosome or a virus, that behaves as an autonomous unit of polynucleotide replication within a cell.

10 A “vector” is a replicon in which another polynucleotide segment is attached, such as to bring about the replication and/or expression of the attached segment.

15 The term “control sequence” refers to a polynucleotide sequence which is necessary to effect the expression of a coding sequence to which it is ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, such control sequences generally include a promoter, a ribosomal binding site and terminators; in eukaryotes, such control sequences generally include promoters, terminators and, in some instances, enhancers. The term “control sequence” thus is intended to include at a minimum all components whose presence is necessary for expression, and also may include additional components whose presence is advantageous, for example, leader sequences.

20 “Operably linked” refers to a situation wherein the components described are in a relationship permitting them to function in their intended manner. Thus, for example, a control sequence “operably linked” to a coding sequence is ligated in such a manner that expression of the coding sequence is achieved under conditions compatible with the control sequence.

25 The term “open reading frame” or “ORF” refers to a region of a polynucleotide sequence which encodes a polypeptide. This region may represent a portion of a coding sequence or a total coding sequence.

30 A “coding sequence” is a polynucleotide sequence which is transcribed into mRNA and translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5' -terminus and a translation stop codon at the 3' -terminus. A coding sequence can include, but is not limited to, mRNA, cDNA and recombinant polynucleotide sequences.

35 The term “immunologically identifiable with/as” refers to the presence of epitope(s) and polypeptide(s) which also are present in and are unique to the designated polypeptide(s). Immunological identity may be determined by antibody

binding and/or competition in binding. These techniques are known to the routineer and also are described herein. The uniqueness of an epitope also can be determined by computer searches of known data banks, such as GenBank, for the polynucleotide sequence which encodes the epitope and by amino acid sequence 5 comparisons with other known proteins.

As used herein, "epitope" means an antigenic determinant of a polypeptide or protein. Conceivably, an epitope can comprise three amino acids in a spatial conformation which is unique to the epitope. Generally, an epitope consists of at least five such amino acids and more usually, it consists of at least eight to ten amino 10 acids. Methods of examining spatial conformation are known in the art and include, for example, x-ray crystallography and two-dimensional nuclear magnetic resonance.

A "conformational epitope" is an epitope that is comprised of a specific juxtaposition of amino acids in an immunologically recognizable structure, such 15 amino acids being present on the same polypeptide in a contiguous or non-contiguous order or present on different polypeptides.

A polypeptide is "immunologically reactive" with an antibody when it binds to an antibody due to antibody recognition of a specific epitope contained within the polypeptide. Immunological reactivity may be determined by antibody binding, 20 more particularly, by the kinetics of antibody binding, and/or by competition in binding using as competitor(s) a known polypeptide(s) containing an epitope against which the antibody is directed. The methods for determining whether a polypeptide is immunologically reactive with an antibody are known in the art.

As used herein, the term "immunogenic polypeptide containing an epitope of 25 interest" means naturally occurring polypeptides of interest or fragments thereof, as well as polypeptides prepared by other means, for example, by chemical synthesis or the expression of the polypeptide in a recombinant organism.

The term "transfection" refers to the introduction of an exogenous polynucleotide into a prokaryotic or eucaryotic host cell, irrespective of the method 30 used for the introduction. The term "transfection" refers to both stable and transient introduction of the polynucleotide, and encompasses direct uptake of polynucleotides, transformation, transduction, and f-mating. Once introduced into the host cell, the exogenous polynucleotide may be maintained as a non-integrated replicon, for example, a plasmid, or alternatively, may be integrated into the host 35 genome.

"Treatment" refers to prophylaxis and/or therapy.

The term "individual" as used herein refers to vertebrates, particularly members of the mammalian species and includes, but is not limited to, domestic animals, sports animals, primates and humans; more particularly, the term refers to humans.

5 The term "sense strand" or "plus strand" (or "+") as used herein denotes a nucleic acid that contains the sequence that encodes the polypeptide. The term "antisense strand" or "minus strand" (or "-") denotes a nucleic acid that contains a sequence that is complementary to that of the "plus" strand.

10 The term "test sample" refers to a component of an individual's body which is the source of the analyte (such as antibodies of interest or antigens of interest). These components are well known in the art. A test sample is typically anything suspected of containing a target sequence. Test samples can be prepared using methodologies well known in the art such as by obtaining a specimen from an individual and, if necessary, disrupting any cells contained thereby to release target 15 nucleic acids. These test samples include biological samples which can be tested by the methods of the present invention described herein and include human and animal body fluids such as whole blood, serum, plasma, cerebrospinal fluid, sputum, bronchial washing, bronchial aspirates, urine, lymph fluids, and various external secretions of the respiratory, intestinal and genitourinary tracts, tears, saliva, milk, 20 white blood cells, myelomas and the like; biological fluids such as cell culture supernatants; tissue specimens which may be fixed; and cell specimens which may be fixed.

25 "Purified product" refers to a preparation of the product which has been isolated from the cellular constituents with which the product is normally associated and from other types of cells which may be present in the sample of interest.

30 "PNA" denotes a "peptide nucleic acid analog" which may be utilized in a procedure such as an assay described herein to determine the presence of a target. "MA" denotes a "morpholino analog" which may be utilized in a procedure such as an assay described herein to determine the presence of a target. See, for example, U.S. Patent No. 5,378,841. PNAs are neutrally charged moieties which can be directed against RNA targets or DNA. PNA probes used in assays in place of, for example, the DNA probes of the present invention, offer advantages not achievable when DNA probes are used. These advantages include manufacturability, large scale labeling, reproducibility, stability, insensitivity to changes in ionic strength and 35 resistance to enzymatic degradation which is present in methods utilizing DNA or RNA. These PNAs can be labeled with ("attached to") such signal generating

compounds as fluorescein, radionucleotides, chemiluminescent compounds and the like. PNAs or other nucleic acid analogs such as MAs thus can be used in assay methods in place of DNA or RNA. Although assays are described herein utilizing DNA probes, it is within the scope of the routineer that PNAs or MAs can be 5 substituted for RNA or DNA with appropriate changes if and as needed in assay reagents.

“Analyte,” as used herein, is the substance to be detected which may be present in the test sample. The analyte can be any substance for which there exists a naturally occurring specific binding member (such as an antibody), or for which a 10 specific binding member can be prepared. Thus, an analyte is a substance that can bind to one or more specific binding members in an assay. “Analyte” also includes any antigenic substances, haptens, antibodies and combinations thereof. As a member of a specific binding pair, the analyte can be detected by means of naturally occurring specific binding partners (pairs) such as the use of intrinsic factor protein 15 as a member of a specific binding pair for the determination of Vitamin B12, the use of folate-binding protein to determine folic acid, or the use of a lectin as a member of a specific binding pair for the determination of a carbohydrate. The analyte can include a protein, a polypeptide, an amino acid, a nucleotide target and the like.

The terms “diseases of the GI tract,” “GI tract disease,” and “condition of the 20 GI tract” are used interchangeably herein to refer to any disease or condition of the esophagus, stomach, small and large intestines, rectum and pancreas including, but not limited to, Barret’s esophagus, gastric ulcer, gastritis, leiomyoma, polyps, Crohn’s disease, ulcerative colitis, pancreatitis and cancer.

“GI tract cancer,” as used herein, refers to any malignant disease of the 25 gastrointestinal tract including, but not limited to, adenocarcinoma, mucinous adenocarcinoma, carcinoid tumor, squamous cell carcinoma, lymphoma, and sarcoma.

An “Expressed Sequence Tag” or “EST” refers to the partial sequence of a 30 cDNA insert which has been made by reverse transcription of mRNA extracted from a tissue followed by insertion into a vector.

A “transcript image” refers to a table or list giving the quantitative distribution of ESTs in a library and represents the genes active in the tissue from which the library was made.

The present invention provides assays which utilize specific binding 35 members. A “specific binding member,” as used herein, is a member of a specific binding pair. That is, two different molecules where one of the molecules, through

chemical or physical means, specifically binds to the second molecule. Therefore, in addition to antigen and antibody specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors, and enzymes and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, antibodies and antibody fragments, both monoclonal and polyclonal and complexes thereof, including those formed by recombinant DNA molecules.

The term "hapten," as used herein, refers to a partial antigen or non-protein binding member which is capable of binding to an antibody, but which is not capable of eliciting antibody formation unless coupled to a carrier protein.

A "capture reagent," as used herein, refers to an unlabeled specific binding member which is specific either for the analyte as in a sandwich assay, for the indicator reagent or analyte as in a competitive assay, or for an ancillary specific binding member, which itself is specific for the analyte, as in an indirect assay. The capture reagent can be directly or indirectly bound to a solid phase material before the performance of the assay or during the performance of the assay, thereby enabling the separation of immobilized complexes from the test sample.

The "indicator reagent" comprises a "signal-generating compound" ("label") which is capable of generating and generates a measurable signal detectable by external means, conjugated ("attached") to a specific binding member. In addition to being an antibody member of a specific binding pair, the indicator reagent also can be a member of any specific binding pair, including either hapten-anti-hapten systems such as biotin or anti-biotin, avidin or biotin, a carbohydrate or a lectin, a complementary nucleotide sequence, an effector or a receptor molecule, an enzyme cofactor and an enzyme, an enzyme inhibitor or an enzyme and the like. An immunoreactive specific binding member can be an antibody, an antigen, or an antibody/antigen complex that is capable of binding either to the polypeptide of interest as in a sandwich assay, to the capture reagent as in a competitive assay, or to the ancillary specific binding member as in an indirect assay. When describing probes and probe assays, the term "reporter molecule" may be used. A reporter molecule comprises a signal generating compound as described hereinabove conjugated to a specific binding member of a specific binding pair, such as carbazole or adamantane.

The various "signal-generating compounds" (labels) contemplated include chromagens, catalysts such as enzymes, luminescent compounds such as fluorescein and rhodamine, chemiluminescent compounds such as dioxetanes, acridiniums, phenanthridiniums and luminol, radioactive elements and direct visual labels.

5 Examples of enzymes include alkaline phosphatase, horseradish peroxidase, beta-galactosidase and the like. The selection of a particular label is not critical, but it must be capable of producing a signal either by itself or in conjunction with one or more additional substances.

"Solid phases" ("solid supports") are known to those in the art and include 10 the walls of wells of a reaction tray, test tubes, polystyrene beads, magnetic or non-magnetic beads, nitrocellulose strips, membranes, microparticles such as latex particles, sheep (or other animal) red blood cells and Duracytes<sup>®</sup> (red blood cells "fixed" by pyruvic aldehyde and formaldehyde, available from Abbott Laboratories, Abbott Park, IL) and others. The "solid phase" is not critical and can be selected by 15 one skilled in the art. Thus, latex particles, microparticles, magnetic or non-magnetic beads, membranes, plastic tubes, walls of microtiter wells, glass or silicon chips, sheep (or other suitable animal's) red blood cells and Duracytes<sup>®</sup> are all suitable examples. Suitable methods for immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like. A "solid phase," as 20 used herein, refers to any material which is insoluble, or can be made insoluble by a subsequent reaction. The solid phase can be chosen for its intrinsic ability to attract and immobilize the capture reagent. Alternatively, the solid phase can retain an additional receptor which has the ability to attract and immobilize the capture reagent. The additional receptor can include a charged substance that is oppositely charged 25 with respect to the capture reagent itself or to a charged substance conjugated to the capture reagent. As yet another alternative, the receptor molecule can be any specific binding member which is immobilized upon (attached to) the solid phase and which has the ability to immobilize the capture reagent through a specific binding reaction. The receptor molecule enables the indirect binding of the capture reagent to a solid 30 phase material before the performance of the assay or during the performance of the assay. The solid phase thus can be a plastic, derivatized plastic, magnetic or non-magnetic metal, glass or silicon surface of a test tube, microtiter well, sheet, bead, microparticle, chip, sheep (or other suitable animal's) red blood cells, Duracytes<sup>®</sup> and other configurations known to those of ordinary skill in the art.

35 It is contemplated and within the scope of the present invention that the solid phase also can comprise any suitable porous material with sufficient porosity to

allow access by detection antibodies and a suitable surface affinity to bind antigens. Microporous structures generally are preferred, but materials with a gel structure in the hydrated state may be used as well. Such useful solid supports include, but are not limited to, nitrocellulose and nylon. It is contemplated that such porous solid

5 supports described herein preferably are in the form of sheets of thickness from about 0.01 to 0.5 mm, preferably about 0.1 mm. The pore size may vary within wide limits and preferably is from about 0.025 to 15 microns, especially from about 0.15 to 15 microns. The surface of such supports may be activated by chemical processes which cause covalent linkage of the antigen or antibody to the support.

10 The irreversible binding of the antigen or antibody is obtained, however, in general, by adsorption on the porous material by poorly understood hydrophobic forces. Other suitable solid supports are known in the art.

Reagents.

The present invention provides reagents such as polynucleotide sequences

15 derived from a GI tract tissue of interest and designated as CS198, polypeptides encoded thereby and antibodies specific for these polypeptides. The present invention also provides reagents such as oligonucleotide fragments derived from the disclosed polynucleotides and nucleic acid sequences complementary to these polynucleotides. The polynucleotides, polypeptides, or antibodies of the present

20 invention may be used to provide information leading to the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating of, or determining the predisposition to, diseases and conditions of the GI tract, such as GI tract cancer. The sequences disclosed herein represent unique polynucleotides which can be used in assays or for producing a specific profile of gene transcription activity. Such

25 assays are disclosed in European Patent Number 0373203B1 and International Publication No. WO 95/11995.

30 Selected CS198-derived polynucleotides can be used in the methods described herein for the detection of normal or altered gene expression. Such methods may employ CS198 polynucleotides or oligonucleotides, fragments or derivatives thereof, or nucleic acid sequences complementary thereto.

35 The polynucleotides disclosed herein, their complementary sequences, or fragments of either, can be used in assays to detect, amplify or quantify genes, nucleic acids, cDNAs or mRNAs relating to GI tract tissue disease and conditions associated therewith. They also can be used to identify an entire or partial coding region of a CS198 polypeptide. They further can be provided in individual containers in the form of a kit for assays, or provided as individual compositions. If

-23-

provided in a kit for assays, other suitable reagents such as buffers, conjugates and the like may be included.

The polynucleotide may be in the form of RNA or DNA. Polynucleotides in the form of DNA, cDNA, genomic DNA, nucleic acid analogs and synthetic DNA 5 are within the scope of the present invention. The DNA may be double-stranded or single-stranded, and if single stranded, may be the coding (sense) strand or non-coding (anti-sense) strand. The coding sequence which encodes the polypeptide may be identical to the coding sequence provided herein or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the 10 genetic code, encodes the same polypeptide as the DNA provided herein.

This polynucleotide may include only the coding sequence for the polypeptide, or the coding sequence for the polypeptide and an additional coding sequence such as a leader or secretory sequence or a proprotein sequence, or the coding sequence for the polypeptide (and optionally an additional coding sequence) 15 and non-coding sequence, such as a non-coding sequence 5' and/or 3' of the coding sequence for the polypeptide.

In addition, the invention includes variant polynucleotides containing modifications such as polynucleotide deletions, substitutions or additions; and any 20 polypeptide modification resulting from the variant polynucleotide sequence. A polynucleotide of the present invention also may have a coding sequence which is a naturally occurring allelic variant of the coding sequence provided herein.

In addition, the coding sequence for the polypeptide may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which 25 functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence is a proprotein and may have the leader sequence cleaved by the host cell to form the polypeptide. The polynucleotides may also encode for a proprotein which is the protein plus additional 5' amino acid residues. A protein having a prosequence is a proprotein and may, in 30 some cases, be an inactive form of the protein. Once the prosequence is cleaved, an active protein remains. Thus, the polynucleotide of the present invention may encode for a protein, or for a protein having a prosequence, or for a protein having both a prosequence (leader sequence) and a prosequence.

The polynucleotides of the present invention may also have the coding 35 sequence fused in frame to a marker sequence which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine

tag supplied by a pQE-9 vector to provide for purification of the polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. a COS-7 cell line, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein. See, for example, I. Wilson et al., Cell 37:767 (1984).

It is contemplated that polynucleotides will be considered to hybridize to the sequences provided herein if there is at least 50%, preferably at least 70%, and more preferably at least 90% identity between the polynucleotide and the sequence.

The present invention also provides an antibody produced by using a purified CS198 polypeptide of which at least a portion of the polypeptide is encoded by a CS198 polynucleotide selected from the polynucleotides provided herein. These antibodies may be used in the methods provided herein for the detection of CS198 antigen in test samples. The presence of CS198 antigen in the test samples is indicative of the presence of a GI tract disease or condition. The antibody also may be used for therapeutic purposes, for example, in neutralizing the activity of CS198 polypeptide in conditions associated with altered or abnormal expression.

The present invention further relates to a CS198 polypeptide which has the deduced amino acid sequence as provided herein, as well as fragments, analogs and derivatives of such polypeptide. The polypeptide of the present invention may be a recombinant polypeptide, a natural purified polypeptide or a synthetic polypeptide. The fragment, derivative or analog of the CS198 polypeptide may be one in which one or more of the amino acid residues is substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code; or it may be one in which one or more of the amino acid residues includes a substituent group; or it may be one in which the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol); or it may be one in which the additional amino acids are fused to the polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are within the scope of the present invention. The polypeptides and polynucleotides of the present invention are provided preferably in an isolated form and preferably purified.

Thus, a polypeptide of the present invention may have an amino acid sequence that is identical to that of the naturally occurring polypeptide or that is different by minor variations due to one or more amino acid substitutions. The

-25-

variation may be a "conservative change" typically in the range of about 1 to 5 amino acids, wherein the substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine or threonine with serine. In contrast, variations may include nonconservative changes, e.g., replacement of a 5 glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted without changing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software (DNASTAR Inc., Madison 10 WI).

Probes constructed according to the polynucleotide sequences of the present invention can be used in various assay methods to provide various types of analysis. For example, such probes can be used in fluorescent *in situ* hybridization (FISH) technology to perform chromosomal analysis, and used to identify cancer-specific 15 structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR-generated and/or allele specific oligonucleotides probes, allele specific amplification or by direct sequencing. Probes also can be labeled with radioisotopes, directly- or indirectly- detectable haptens, or fluorescent molecules, and utilized for *in situ* hybridization 20 studies to evaluate the mRNA expression of the gene comprising the polynucleotide in tissue specimens or cells.

This invention also provides teachings as to the production of the polynucleotides and polypeptides provided herein.

#### Probe Assays

25 The sequences provided herein may be used to produce probes which can be used in assays for the detection of nucleic acids in test samples. The probes may be designed from conserved nucleotide regions of the polynucleotides of interest or from non-conserved nucleotide regions of the polynucleotide of interest. The design of such probes for optimization in assays is within the skill of the routineer.

30 Generally, nucleic acid probes are developed from non-conserved or unique regions when maximum specificity is desired, and nucleic acid probes are developed from conserved regions when assaying for nucleotide regions that are closely related to, for example, different members of a multi-gene family or in related species like mouse and man.

35 The polymerase chain reaction (PCR) is a technique for amplifying a desired nucleic acid sequence (target) contained in a nucleic acid or mixture thereof. In

PCR, a pair of primers are employed in excess to hybridize to the complementary strands of the target nucleic acid. The primers are each extended by a polymerase using the target nucleic acid as a template. The extension products become target sequences themselves, following dissociation from the original target strand. New 5 primers then are hybridized and extended by a polymerase, and the cycle is repeated to geometrically increase the number of target sequence molecules. PCR is disclosed in U.S. Patent Nos 4,683,195 and 4,683,202.

The Ligase Chain Reaction (LCR) is an alternate method for nucleic acid amplification. In LCR, probe pairs are used which include two primary (first and 10 second) and two secondary (third and fourth) probes, all of which are employed in molar excess to target. The first probe hybridizes to a first segment of the target strand, and the second probe hybridizes to a second segment of the target strand, the first and second segments being contiguous so that the primary probes abut one another in 5' phosphate-3' hydroxyl relationship, and so that a ligase can covalently 15 fuse or ligate the two probes into a fused product. In addition, a third (secondary) probe can hybridize to a portion of the first probe and a fourth (secondary) probe can hybridize to a portion of the second probe in a similar abutting fashion. Of course, if the target is initially double stranded, the secondary probes also will hybridize to the target complement in the first instance. Once the ligated strand of primary probes 20 is separated from the target strand, it will hybridize with the third and fourth probes which can be ligated to form a complementary, secondary ligated product. It is important to realize that the ligated products are functionally equivalent to either the target or its complement. By repeated cycles of hybridization and ligation, amplification of the target sequence is achieved. This technique is described more 25 completely in EP-A- 320 308 to K. Backman published June 16, 1989 and EP-A- 439 182 to K. Backman et al, published July 31, 1991.

For amplification of mRNAs, it is within the scope of the present invention to reverse transcribe mRNA into cDNA followed by polymerase chain reaction (RT-PCR); or, to use a single enzyme for both steps as described in U.S. Patent No. 30 5,322,770; or reverse transcribe mRNA into cDNA followed by asymmetric gap ligase chain reaction (RT-AGLCR) as described by R.L. Marshall et al., PCR Methods and Applications 4: 80-84 (1994).

Other known amplification methods which can be utilized herein include but are not limited to the so-called "NASBA" or "3SR" technique described by J.C. 35 Guatelli et al., PNAS USA 87:1874-1878 (1990) and also described by J. Compton, Nature 350 (No. 6313):91-92 (1991); Q-beta amplification as described

in published European Patent Application (EPA) No. 4544610; strand displacement amplification (as described in G.T. Walker et al., Clin. Chem. 42:9-13 [1996]) and European Patent Application No. 684315; and target mediated amplification, as described in International Publication No. WO 93/22461.

5       Detection of CS198 may be accomplished using any suitable detection method, including those detection methods which are currently well known in the art, as well as detection strategies which may evolve later. See, for example, Caskey et al., U.S. Patent No. 5,582,989, Gelfand et al., U.S. Patent No. 5,210,015. Examples of such detection methods include target amplification 10 methods as well as signal amplification technologies. An example of presently known detection methods would include the nucleic acid amplification technologies referred to as PCR, LCR, NASBA, SDA, RCR and TMA. See, for example, Caskey et al., U.S. Patent No. 5,582,989, Gelfand et al., U.S. Patent No. 5,210,015. Detection may also be accomplished using signal amplification such as 15 that disclosed in Snitman et al., U.S. Patent No. 5,273,882. While the amplification of target or signal is preferred at present, it is contemplated and within the scope of the present invention that ultrasensitive detection methods which do not require amplification can be utilized herein.

20       Detection, both amplified and non-amplified, may be (combined) carried out using a variety of heterogeneous and homogeneous detection formats. Examples of heterogeneous detection formats are disclosed in Snitman et al., U.S. Patent No. 5,273,882, Albarella et al in EP-84114441.9, Urdea et al., U.S. Patent No. 5,124,246, Ullman et al. U.S. Patent No. 5,185,243 and Kourilsky et al., U.S. Patent No. 4,581,333. Examples of homogeneous detection formats are disclosed 25 in, Caskey et al., U.S. Patent No. 5,582,989, Gelfand et al., U.S. Patent No. 5,210,015. Also contemplated and within the scope of the present invention is the use of multiple probes in the hybridization assay, which use improves sensitivity and amplification of the CS198 signal. See, for example, Caskey et al., U.S. Patent No. 5,582,989, and Gelfand et al., U.S. Patent No. 5,210,015.

30       In one embodiment, the present invention generally comprises the steps of contacting a test sample suspected of containing a target polynucleotide sequence with amplification reaction reagents comprising an amplification primer, and a detection probe that can hybridize with an internal region of the amplicon sequences. Probes and primers employed according to the method provided herein are labeled 35 with capture and detection labels, wherein probes are labeled with one type of label and primers are labeled with another type of label. Additionally, the primers and

probes are selected such that the probe sequence has a lower melt temperature than the primer sequences. The amplification reagents, detection reagents and test sample are placed under amplification conditions whereby, in the presence of target sequence, copies of the target sequence (an amplicon) are produced. In the usual 5 case, the amplicon is double stranded because primers are provided to amplify a target sequence and its complementary strand. The double stranded amplicon then is thermally denatured to produce single stranded amplicon members. Upon formation of the single stranded amplicon members, the mixture is cooled to allow the formation of complexes between the probes and single stranded amplicon members.

10 As the single stranded amplicon sequences and probe sequences are cooled, the probe sequences preferentially bind the single stranded amplicon members. This finding is counterintuitive given that the probe sequences generally are selected to be shorter than the primer sequences and therefore have a lower melt temperature than the primers. Accordingly, the melt temperature of the amplicon produced by the 15 primers should also have a higher melt temperature than the probes. Thus, as the mixture cools, the re-formation of the double stranded amplicon would be expected. As previously stated, however, this is not the case. The probes are found to preferentially bind the single stranded amplicon members. Moreover, this 20 preference of probe/single stranded amplicon binding exists even when the primer sequences are added in excess of the probes.

After the probe/single stranded amplicon member hybrids are formed, they are detected. Standard heterogeneous assay formats are suitable for detecting the hybrids using the detection labels and capture labels present on the primers and probes. The hybrids can be bound to a solid phase reagent by virtue of the capture 25 label and detected by virtue of the detection label. In cases where the detection label is directly detectable, the presence of the hybrids on the solid phase can be detected by causing the label to produce a detectable signal, if necessary, and detecting the signal. In cases where the label is not directly detectable, the captured hybrids can be contacted with a conjugate, which generally comprises a binding member attached 30 to a directly detectable label. The conjugate becomes bound to the complexes and the conjugate's presence on the complexes can be detected with the directly detectable label. Thus, the presence of the hybrids on the solid phase reagent can be determined. Those skilled in the art will recognize that wash steps may be employed to wash away unhybridized amplicon or probe as well as unbound conjugate.

35 Although the target sequence is described as single stranded, it also is contemplated to include the case where the target sequence is actually double

stranded but is merely separated from its complement prior to hybridization with the amplification primer sequences. In the case where PCR is employed in this method, the ends of the target sequences are usually known. In cases where LCR or a modification thereof is employed in the preferred method, the entire target sequence 5 is usually known. Typically, the target sequence is a nucleic acid sequence such as, for example, RNA or DNA.

The method provided herein can be used in well-known amplification reactions that include thermal cycle reaction mixtures, particularly in PCR and gap LCR (GLCR). Amplification reactions typically employ primers to repeatedly 10 generate copies of a target nucleic acid sequence, which target sequence is usually a small region of a much larger nucleic acid sequence. Primers are themselves nucleic acid sequences that are complementary to regions of a target sequence. Under amplification conditions, these primers hybridize or bind to the complementary regions of the target sequence. Copies of the target sequence typically are generated 15 by the process of primer extension and/or ligation which utilizes enzymes with polymerase or ligase activity, separately or in combination, to add nucleotides to the hybridized primers and/or ligate adjacent probe pairs. The nucleotides that are added to the primers or probes, as monomers or preformed oligomers, are also complementary to the target sequence. Once the primers or probes have been 20 sufficiently extended and/or ligated, they are separated from the target sequence, for example, by heating the reaction mixture to a "melt temperature" which is one in which complementary nucleic acid strands dissociate. Thus, a sequence complementary to the target sequence is formed.

A new amplification cycle then can take place to further amplify the number 25 of target sequences by separating any double stranded sequences, allowing primers or probes to hybridize to their respective targets, extending and/or ligating the hybridized primers or probes and re-separating. The complementary sequences that are generated by amplification cycles can serve as templates for primer extension or filling the gap of two probes to further amplify the number of target sequences. 30 Typically, a reaction mixture is cycled between 20 and 100 times, more typically, a reaction mixture is cycled between 25 and 50 times. The numbers of cycles can be determined by the routineer. In this manner, multiple copies of the target sequence and its complementary sequence are produced. Thus, primers initiate amplification of the target sequence when it is present under amplification conditions.

35 Generally, two primers which are complementary to a portion of a target strand and its complement are employed in PCR. For LCR, four probes, two of

which are complementary to a target sequence and two of which are similarly complementary to the target's complement, are generally employed. In addition to the primer sets and enzymes previously mentioned, a nucleic acid amplification reaction mixture may also comprise other reagents which are well known and 5 include but are not limited to: enzyme cofactors such as manganese; magnesium; salts; nicotinamide adenine dinucleotide (NAD); and deoxynucleotide triphosphates (dNTPs) such as, for example, deoxyadenine triphosphate, deoxyguanine triphosphate, deoxycytosine triphosphate and deoxythymine triphosphate.

While the amplification primers initiate amplification of the target sequence, 10 the detection (or hybridization) probe is not involved in amplification. Detection probes are generally nucleic acid sequences or uncharged nucleic acid analogs such as, for example, peptide nucleic acids which are disclosed in International Publication No. WO 92/20702; morpholino analogs which are described in U.S. Patents Nos 5,185,444, 5,034,506 and 5,142,047; and the like. Depending upon 15 the type of label carried by the probe, the probe is employed to capture or detect the amplicon generated by the amplification reaction. The probe is not involved in amplification of the target sequence and therefore may have to be rendered "non-extendible" in that additional dNTPs cannot be added to the probe. In and of themselves, analogs usually are non-extendible and nucleic acid probes can be 20 rendered non-extendible by modifying the 3' end of the probe such that the hydroxyl group is no longer capable of participating in elongation. For example, the 3' end of the probe can be functionalized with the capture or detection label to thereby consume or otherwise block the hydroxyl group. Alternatively, the 3' hydroxyl group simply can be cleaved, replaced or modified. U.S. Patent Application Serial 25 No. 07/049,061 filed April 19, 1993 describes modifications which can be used to render a probe non-extendible.

The ratio of primers to probes is not important. Thus, either the probes or 30 primers can be added to the reaction mixture in excess whereby the concentration of one would be greater than the concentration of the other. Alternatively, primers and probes can be employed in equivalent concentrations. Preferably, however, the primers are added to the reaction mixture in excess of the probes. Thus, primer to probe ratios of, for example, 5:1 and 20:1, are preferred.

While the length of the primers and probes can vary, the probe sequences are selected such that they have a lower melt temperature than the primer sequences. 35 Hence, the primer sequences are generally longer than the probe sequences. Typically, the primer sequences are in the range of between 20 and 50 nucleotides

long, more typically in the range of between 20 and 30 nucleotides long. The typical probe is in the range of between 10 and 25 nucleotides long.

Various methods for synthesizing primers and probes are well known in the art. Similarly, methods for attaching labels to primers or probes are also well known in the art. For example, it is routine to synthesize desired nucleic acid primers or probes using conventional nucleotide phosphoramidite chemistry and instruments available from Applied Biosystems, Inc., (Foster City, CA), DuPont (Wilmington, DE), or Milligen (Bedford MA). Many methods have been described for labeling oligonucleotides such as the primers or probes of the present invention.

5 Enzo Biochemical (New York, NY) and Clontech (Palo Alto, CA) both have described and commercialized probe labeling techniques. For example, a primary amine can be attached to a 3' oligo terminus using 3'-Amine-ON CPG™ (Clontech, Palo Alto, CA). Similarly, a primary amine can be attached to a 5' oligo terminus using Aminomodifier II® (Clontech). The amines can be reacted to various haptens

10 using conventional activation and linking chemistries. In addition, copending applications U.S. Serial Nos. 625,566, filed December 11, 1990 and 630,908, filed December 20, 1990, teach methods for labeling probes at their 5' and 3' termini, respectively. International Publication Nos WO 92/10505, published 25 June 1992, and WO 92/11388, published 9 July 1992, teach methods for labeling probes at their

15 5' and 3' ends, respectively. According to one known method for labeling an oligonucleotide, a label-phosphoramidite reagent is prepared and used to add the label to the oligonucleotide during its synthesis. See, for example, N.T. Thuong et al., Tet. Letters 29(46):5905-5908 (1988); or J.S. Cohen et al., published U.S. Patent Application 07/246,688 (NTIS ORDER No. PAT-APPL-7-246,688) (1989).

20 Preferably, probes are labeled at their 3' and 5' ends.

A capture label is attached to the primers or probes and can be a specific binding member which forms a binding pair with the solid phase reagent's specific binding member. It will be understood that the primer or probe itself may serve as the capture label. For example, in the case where a solid phase reagent's binding

25 member is a nucleic acid sequence, it may be selected such that it binds a complementary portion of the primer or probe to thereby immobilize the primer or probe to the solid phase. In cases where the probe itself serves as the binding member, those skilled in the art will recognize that the probe will contain a sequence or "tail" that is not complementary to the single stranded amplicon members. In the case where the primer itself serves as the capture label, at least a portion of the

primer will be free to hybridize with a nucleic acid on a solid phase because the probe is selected such that it is not fully complementary to the primer sequence.

Generally, probe/single stranded amplicon member complexes can be detected using techniques commonly employed to perform heterogeneous immunoassays. Preferably, in this embodiment, detection is performed according to the protocols used by the commercially available Abbott LCx® instrumentation (Abbott Laboratories, Abbott Park, IL).

The primers and probes disclosed herein are useful in typical PCR assays, wherein the test sample is contacted with a pair of primers, amplification is performed, the hybridization probe is added, and detection is performed.

Another method provided by the present invention comprises contacting a test sample with a plurality of polynucleotides, wherein at least one polynucleotide is a CS198 molecule as described herein, hybridizing the test sample with the plurality of polynucleotides and detecting hybridization complexes. Hybridization complexes are identified and quantitated to compile a profile which is indicative of GI tract tissue disease, such as GI tract cancer. Expressed RNA sequences may further be detected by reverse transcription and amplification of the DNA product by procedures well-known in the art, including polymerase chain reaction (PCR).

#### Drug Screening and Gene Therapy.

The present invention also encompasses the use of gene therapy methods for the introduction of anti-sense CS198 derived molecules, such as polynucleotides or oligonucleotides of the present invention, into patients with conditions associated with abnormal expression of polynucleotides related to a GI tract tissue disease or condition especially GI tract cancer. These molecules, including antisense RNA and DNA fragments and ribozymes, are designed to inhibit the translation of CS198-mRNA, and may be used therapeutically in the treatment of conditions associated with altered or abnormal expression of CS198 polynucleotide.

Alternatively, the oligonucleotides described above can be delivered to cells by procedures known in the art such that the anti-sense RNA or DNA may be expressed in vivo to inhibit production of a CS198 polypeptide in the manner described above. Antisense constructs to a CS198 polynucleotide, therefore, reverse the action of CS198 transcripts and may be used for treating GI tract tissue disease conditions, such as GI tract cancer. These antisense constructs may also be used to treat tumor metastases.

The present invention also provides a method of screening a plurality of compounds for specific binding to CS198 polypeptide(s), or any fragment thereof,

to identify at least one compound which specifically binds the CS198 polypeptide. Such a method comprises the steps of providing at least one compound; combining the CS198 polypeptide with each compound under suitable conditions for a time sufficient to allow binding; and detecting the CS198 polypeptide binding to each 5 compound.

The polypeptide or peptide fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. One method of screening utilizes eukaryotic or prokaryotic host cells which are stably transfected with recombinant nucleic acids which can express the 10 polypeptide or peptide fragment. A drug, compound, or any other agent may be screened against such transfected cells in competitive binding assays. For example, the formation of complexes between a polypeptide and the agent being tested can be measured in either viable or fixed cells.

The present invention thus provides methods of screening for drugs, 15 compounds, or any other agent which can be used to treat diseases associated with CS198. These methods comprise contacting the agent with a polypeptide or fragment thereof and assaying for either the presence of a complex between the agent and the polypeptide, or for the presence of a complex between the polypeptide and the cell. In competitive binding assays, the polypeptide typically is labeled. After 20 suitable incubation, free (or uncomplexed) polypeptide or fragment thereof is separated from that present in bound form, and the amount of free or uncomplexed label is used as a measure of the ability of the particular agent to bind to the polypeptide or to interfere with the polypeptide/cell complex.

The present invention also encompasses the use of competitive screening 25 assays in which neutralizing antibodies capable of binding polypeptide specifically compete with a test agent for binding to the polypeptide or fragment thereof. In this manner, the antibodies can be used to detect the presence of any polypeptide in the test sample which shares one or more antigenic determinants with a CS198 polypeptide as provided herein.

Another technique for screening provides high throughput screening for 30 compounds having suitable binding affinity to at least one polypeptide of CS198 disclosed herein. Briefly, large numbers of different small peptide test compounds are synthesized on a solid phase, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptide and washed. Polypeptide thus 35 bound to the solid phase is detected by methods well-known in the art. Purified polypeptide can also be coated directly onto plates for use in the screening

-34-

techniques described herein. In addition, non-neutralizing antibodies can be used to capture the polypeptide and immobilize it on the solid support. See, for example, EP 84/03564, published on September 13, 1984.

The goal of rational drug design is to produce structural analogs of 5 biologically active polypeptides of interest or of the small molecules including agonists, antagonists, or inhibitors with which they interact. Such structural analogs can be used to design drugs which are more active or stable forms of the polypeptide or which enhance or interfere with the function of a polypeptide *in vivo*. J. Hodgson, Bio/Technology 9:19-21 (1991).

10 For example, in one approach, the three-dimensional structure of a polypeptide, or of a polypeptide-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, 15 useful information regarding the structure of a polypeptide may be gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous polypeptide-like molecules or to identify efficient inhibitors

20 Useful examples of rational drug design may include molecules which have improved activity or stability as shown by S. Braxton et al., Biochemistry 31:7796-7801 (1992), or which act as inhibitors, agonists, or antagonists of native peptides as shown by S.B.P. Athauda et al., J Biochem. (Tokyo) 113 (6):742-746 (1993).

25 It also is possible to isolate a target-specific antibody selected by an assay as described hereinabove, and then to determine its crystal structure. In principle this approach yields a pharmacophore upon which subsequent drug design can be based. It further is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies ("anti-ids") to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-id is an analog of the original receptor. The anti-id then can be used to identify and isolate peptides 30 from banks of chemically or biologically produced peptides. The isolated peptides then can act as the pharmacophore (that is, a prototype pharmaceutical drug).

35 A sufficient amount of a recombinant polypeptide of the present invention may be made available to perform analytical studies such as X-ray crystallography. In addition, knowledge of the polypeptide amino acid sequence which is derivable from the nucleic acid sequence provided herein will provide guidance to those

employing computer modeling techniques in place of, or in addition to, x-ray crystallography.

Antibodies specific to a CS198 polypeptide (e.g., anti-CS198 antibodies) further may be used to inhibit the biological action of the polypeptide by binding to 5 the polypeptide. In this manner, the antibodies may be used in therapy, for example, to treat GI tract tissue diseases including GI tract cancer and its metastases.

Further, such antibodies can detect the presence or absence of a CS198 polypeptide in a test sample and, therefore, are useful as diagnostic markers for the diagnosis of a GI tract tissue disease or condition especially GI tract cancer. Such 10 antibodies may also function as a diagnostic marker for GI tract tissue disease conditions, such as GI tract cancer.

The present invention also is directed to antagonists and inhibitors of the polypeptides of the present invention. The antagonists and inhibitors are those which inhibit or eliminate the function of the polypeptide. Thus, for example, an 15 antagonist may bind to a polypeptide of the present invention and inhibit or eliminate its function. The antagonist, for example, could be an antibody against the polypeptide which eliminates the activity of a CS198 polypeptide by binding a CS198 polypeptide, or in some cases the antagonist may be an oligonucleotide. Examples of small molecule inhibitors include, but are not limited to, small peptides 20 or peptide-like molecules.

The antagonists and inhibitors may be employed as a composition with a pharmaceutically acceptable carrier including, but not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. Administration of CS198 polypeptide inhibitors is preferably systemic. The present invention also 25 provides an antibody which inhibits the action of such a polypeptide.

Antisense technology can be used to reduce gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the polypeptide of the present 30 invention, is used to design an antisense RNA oligonucleotide of from 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription, thereby preventing transcription and the production of the CS198 polypeptide. For triple helix, see, for example, Lee et al., Nuc. Acids Res. 6:3073 (1979); Cooney et al, Science 241:456 (1988); and Dervan 35 et al, Science 251:1360 (1991). The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of a mRNA molecule into the CS198

polypeptide. For antisense, see, for example, Okano, *J. Neurochem.* 56:560 (1991); and "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression," CRC Press, Boca Raton, Fla. (1988). Antisense oligonucleotides act with greater efficacy when modified to contain artificial internucleotide linkages which render the 5 molecule resistant to nucleolytic cleavage. Such artificial internucleotide linkages include, but are not limited to, methylphosphonate, phosphorothiolate and phosphoroamidate internucleotide linkages.

Recombinant Technology.

The present invention provides host cells and expression vectors comprising 10 CS198 polynucleotides of the present invention and methods for the production of the polypeptide(s) they encode. Such methods comprise culturing the host cells under conditions suitable for the expression of the CS198 polynucleotide and recovering the CS198 polypeptide from the cell culture.

The present invention also provides vectors which include CS198 15 polynucleotides of the present invention, host cells which are genetically engineered with vectors of the present invention and the production of polypeptides of the present invention by recombinant techniques.

Host cells are genetically engineered (transfected, transduced or transformed) with the vectors of this invention which may be cloning vectors or expression 20 vectors. The vector may be in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transfected cells, or amplifying CS198 gene(s). The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent 25 to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing a polypeptide by recombinant techniques. Thus, the polynucleotide sequence may be included in any one of a variety of expression vehicles, in particular, vectors or plasmids for expressing a polypeptide. Such vectors include chromosomal, 30 nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus and pseudorabies. However, any other plasmid or vector may be used so long as it is replicable and viable in the host.

35 The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into appropriate restriction

endonuclease sites by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art. The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. Representative examples of such 5 promoters include, but are not limited to, the LTR or the SV40 promoter, the E. coli lac or trp, the phage lambda P sub L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for 10 amplifying expression. In addition, the expression vectors preferably contain a gene to provide a phenotypic trait for selection of transfected host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove 15 described, as well as an appropriate promoter or control sequence, may be employed to transfet an appropriate host to permit the host to express the protein. As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Salmonella typhimurium; Streptomyces sp.; fungal cells, such as yeast; insect cells, such as Drosophila and Sf9; animal cells, such as CHO, COS 20 or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings provided herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a 25 sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available. The following vectors are provided by way of 30 example. Bacterial: pINCY (Incyte Pharmaceuticals Inc., Palo Alto, CA), pSPORT1 (Life Technologies, Gaithersburg, MD), pQE70, pQE60, pQE-9 (Qiagen) pBs, phagescript, psiX174, pBluescript SK, pBsKS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRITS (Pharmacia); Eukaryotic: pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene) 35 pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as it is replicable and viable in the host.

Plasmid pINCY is generally identical to the plasmid pSPORT1 (available from Life Technologies, Gaithersburg, MD) with the exception that it has two modifications in the polylinker (multiple cloning site). These modifications are (1) it lacks a HindIII restriction site and (2) its EcoRI restriction site lies at a different location. pINCY is created from pSPORT1 by cleaving pSPORT1 with both HindIII and EcoRI and replacing the excised fragment of the polylinker with synthetic DNA fragments (SEQUENCE ID NO 28 and SEQUENCE ID NO 29). This replacement may be made in any manner known to those of ordinary skill in the art. For example, the two nucleotide sequences, SEQUENCE ID NO 28 and SEQUENCE ID NO 29, may be generated synthetically with 5' terminal phosphates, mixed together, and then ligated under standard conditions for performing staggered end ligations into the pSPORT1 plasmid cut with HindIII and EcoRI. Suitable host cells (such as *E. coli* DH5 $\mu$  cells) then are transfected with the ligated DNA and recombinant clones are selected for ampicillin resistance. Plasmid DNA then is prepared from individual clones and subjected to restriction enzyme analysis or DNA sequencing in order to confirm the presence of insert sequences in the proper orientation. Other cloning strategies known to the ordinary artisan also may be employed.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, SP6, T7, gpt, lambda P sub R, P sub L and trp. Eukaryotic promoters include cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, early and late SV40, LTRs from retroviruses and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention provides host cells containing the above-described construct. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (L. Davis et al., "Basic Methods in Molecular Biology," 2nd edition, Appleton and Lang, Paramount Publishing, East Norwalk, CT (1994)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the

polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Recombinant proteins can be expressed in mammalian cells, yeast, bacteria, or other cells, under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, (Cold Spring Harbor, NY, 1989).

Transcription of a DNA encoding the polypeptide(s) of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin (bp 100 to 270), a cytomegalovirus early promoter enhancer, a polyoma enhancer on the late side of the replication origin and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transfection of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), alpha factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transfection include E. coli, Bacillus subtilis, Salmonella typhimurium and various

species within the genera Pseudomonas, Streptomyces and Staphylococcus, although others may also be employed as a routine matter of choice.

Useful expression vectors for bacterial use comprise a selectable marker and bacterial origin of replication derived from plasmids comprising genetic elements of the well-known cloning vector pBR322 (ATCC 37017). Other vectors include but are not limited to PKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transfection of a suitable host and growth of the host to an appropriate cell density, the selected promoter is derepressed by appropriate means (e.g., temperature shift or chemical induction), and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well-known to the ordinary artisan.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-20 lines of monkey kidney fibroblasts described by Gluzman, Cell 23:175 (1981), and other cell lines capable of expressing a compatible vector, such as the C127, HEK-293, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer and also any necessary ribosome binding sites, polyadenylation sites, splice donor and acceptor sites, transcriptional termination sequences and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Representative, useful vectors include pRc/CMV and pcDNA3 (available from Invitrogen, San Diego, CA).

CS198 polypeptides are recovered and purified from recombinant cell cultures by known methods including affinity chromatography, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxyapatite chromatography or lectin chromatography. It is preferred to have low concentrations (approximately 0.1-5 mM) of calcium ion present during purification (Price, et al., J. Biol. Chem. 244:917 (1969)). Protein refolding steps

-43-

convenient restriction endonuclease digestion sites and selectable markers appropriate for particular host cells. The vectors can be transferred by a variety of means known to those of skill in the art into suitable host cells which then produce the desired DNA, RNA or polypeptides.

5        Occasionally, sequencing or random reverse transcription errors will mask the presence of the appropriate open reading frame or regulatory element. In such cases, it is possible to determine the correct reading frame by attempting to express the polypeptide and determining the amino acid sequence by standard peptide mapping and sequencing techniques. See, F.M. Ausubel et al., Current Protocols in  
10 Molecular Biology, John Wiley & Sons, New York, NY (1989). Additionally, the actual reading frame of a given nucleotide sequence may be determined by transfection of host cells with vectors containing all three potential reading frames. Only those cells with the nucleotide sequence in the correct reading frame will produce a peptide of the predicted length.

15        The nucleotide sequences provided herein have been prepared by current, state-of-the-art, automated methods and, as such, may contain unidentified nucleotides. These will not present a problem to those skilled in the art who wish to practice the invention. Several methods employing standard recombinant techniques, described in J. Sambrook (supra) or periodic updates thereof, may be  
20 used to complete the missing sequence information. The same techniques used for obtaining a full length sequence, as described herein, may be used to obtain nucleotide sequences.

25        Expression of a particular cDNA may be accomplished by subcloning the cDNA into an appropriate expression vector and transfecting this vector into an appropriate expression host. The cloning vector used for the generation of the GI tract tissue cDNA library can be used for transcribing mRNA of a particular cDNA and contains a promoter for beta-galactosidase, an amino-terminal met and the subsequent seven amino acid residues of beta-galactosidase. Immediately following these eight residues is an engineered bacteriophage promoter useful for artificial  
30 priming and transcription, as well as a number of unique restriction sites, including EcoRI, for cloning. The vector can be transfected into an appropriate host strain of E. coli.

35        Induction of the isolated bacterial strain with isopropylthiogalactoside (IPTG) using standard methods will produce a fusion protein which contains the first seven residues of beta-galactosidase, about 15 residues of linker and the peptide encoded within the cDNA. Since cDNA clone inserts are generated by an essentially

random process, there is one chance in three that the included cDNA will lie in the correct frame for proper translation. If the cDNA is not in the proper reading frame, the correct frame can be obtained by deletion or insertion of an appropriate number of bases by well known methods including in vitro mutagenesis, digestion with

5 exonuclease III or mung bean nuclease, or oligonucleotide linker inclusion.

The cDNA can be shuttled into other vectors known to be useful for expression of protein in specific hosts. Oligonucleotide primers containing cloning sites and segments of DNA sufficient to hybridize to stretches at both ends of the target cDNA can be synthesized chemically by standard methods. These primers can

10 then be used to amplify the desired gene segments by PCR. The resulting new gene segments can be digested with appropriate restriction enzymes under standard conditions and isolated by gel electrophoresis. Alternately, similar gene segments can be produced by digestion of the cDNA with appropriate restriction enzymes and filling in the missing gene segments with chemically synthesized oligonucleotides.

15 Segments of the coding sequence from more than one gene can be ligated together and cloned in appropriate vectors to optimize expression of recombinant sequence.

Suitable expression hosts for such chimeric molecules include, but are not limited to, mammalian cells, such as Chinese Hamster Ovary (CHO) and human embryonic kidney (HEK) 293 cells, insect cells, such as Sf9 cells, yeast cells, such

20 as Saccharomyces cerevisiae and bacteria, such as E. coli. For each of these cell systems, a useful expression vector may also include an origin of replication to allow propagation in bacteria and a selectable marker such as the beta-lactamase antibiotic resistance gene to allow selection in bacteria. In addition, the vectors may include a second selectable marker, such as the neomycin phosphotransferase gene,

25 to allow selection in transfected eukaryotic host cells. Vectors for use in eukaryotic expression hosts may require the addition of 3' poly A tail if the sequence of interest lacks poly A.

Additionally, the vector may contain promoters or enhancers which increase gene expression. Such promoters are host specific and include, but are not limited

30 to, MMTV, SV40, or metallothionein promoters for CHO cells; trp, lac, tac or T7 promoters for bacterial hosts; or alpha factor, alcohol oxidase or PGH promoters for yeast. Adenoviral vectors with or without transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to drive protein expression in mammalian cell lines. Once homogeneous cultures of recombinant cells are

35 obtained, large quantities of recombinantly produced protein can be recovered from the conditioned medium and analyzed using chromatographic methods well known

-41-

can be used, as necessary, in completing configuration of the polypeptide. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

Thus, polypeptides of the present invention may be naturally purified products expressed from a high expressing cell line, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated with mammalian or other eukaryotic carbohydrates or may be non-glycosylated. The polypeptides of the invention may also include an initial methionine amino acid residue.

The starting plasmids can be constructed from available plasmids in accord with published, known procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to one of ordinary skill in the art.

The following is the general procedure for the isolation and analysis of cDNA clones. In a particular embodiment disclosed herein, mRNA was isolated from GI tract tissue and used to generate the cDNA library. GI tract tissue was obtained from patients by surgical resection and was classified as tumor or non-tumor tissue by a pathologist.

The cDNA inserts from random isolates of the GI tract tissue libraries were sequenced in part, analyzed in detail as set forth in the Examples, and are disclosed in the Sequence Listing as SEQUENCE ID NOS 7-25. Also analyzed in detail as set forth in the Examples, and disclosed in the Sequence Listing, are the sequences of exons derived from genomic clone g2804590 (SEQUENCE ID NOS 1-6), and the full-length sequence of clone 2055371 (referred to as clone 2055371IH (SEQUENCE ID NO 26)). The consensus sequence of these inserts is presented as SEQUENCE ID NO 27. These polynucleotides may contain an entire open reading frame with or without associated regulatory sequences for a particular gene, or they may encode only a portion of the gene of interest. This is attributed to the fact that many genes are several hundred and sometimes several thousand bases in length and, with current technology, cannot be cloned in their entirety because of vector limitations, incomplete reverse transcription of the first strand, or incomplete replication of the second strand. Contiguous, secondary clones containing additional nucleotide sequences may be obtained using a variety of methods known to those of skill in the art.

Methods for DNA sequencing are well known in the art. Conventional enzymatic methods employ DNA polymerase, Klenow fragment, Sequenase (US Biochemical Corp, Cleveland, OH) or Taq polymerase to extend DNA chains from an oligonucleotide primer annealed to the DNA template of interest. Methods have 5 been developed for the use of both single-stranded and double-stranded templates. The chain termination reaction products may be electrophoresed on urea/polyacrylamide gels and detected either by autoradiography (for radionucleotide labeled precursors) or by fluorescence (for fluorescent-labeled precursors). Recent 10 improvements in mechanized reaction preparation, sequencing and analysis using the fluorescent detection method have permitted expansion in the number of sequences that can be determined per day using machines such as the Applied Biosystems 377 DNA Sequencers (Applied Biosystems, Foster City, CA).

The reading frame of the nucleotide sequence can be ascertained by several 15 types of analyses. First, reading frames contained within the coding sequence can be analyzed for the presence of start codon ATG and stop codons TGA, TAA or TAG. Typically, one reading frame will continue throughout the major portion of a cDNA sequence while other reading frames tend to contain numerous stop codons. In such cases, reading frame determination is straightforward. In other more 20 difficult cases, further analysis is required.

Algorithms have been created to analyze the occurrence of individual 25 nucleotide bases at each putative codon triplet. See, for example J.W. Fickett, Nuc. Acids Res. 10:5303 (1982). Coding DNA for particular organisms (bacteria, plants and animals) tends to contain certain nucleotides within certain triplet periodicities, such as a significant preference for pyrimidines in the third codon position. These 30 preferences have been incorporated into widely available software which can be used to determine coding potential (and frame) of a given stretch of DNA. The algorithm-derived information combined with start/stop codon information can be used to determine proper frame with a high degree of certainty. This, in turn, readily permits cloning of the sequence in the correct reading frame into appropriate expression vectors.

The nucleic acid sequences disclosed herein may be joined to a variety of other polynucleotide sequences and vectors of interest by means of well-established 35 recombinant DNA techniques. See J. Sambrook et al., supra. Vectors of interest include cloning vectors, such as plasmids, cosmids, phage derivatives, phagemids, as well as sequencing, replication and expression vectors, and the like. In general, such vectors contain an origin of replication functional in at least one organism,

in the art. An alternative method for the production of large amounts of secreted protein involves the transfection of mammalian embryos and the recovery of the recombinant protein from milk produced by transgenic cows, goats, sheep, etc. Polypeptides and closely related molecules may be expressed recombinantly in such 5 a way as to facilitate protein purification. One approach involves expression of a chimeric protein which includes one or more additional polypeptide domains not naturally present on human polypeptides. Such purification-facilitating domains include, but are not limited to, metal-chelating peptides such as histidine-tryptophan domains that allow purification on immobilized metals, protein A domains that allow 10 purification on immobilized immunoglobulin and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, WA). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase from Invitrogen (San Diego, CA) between the polypeptide sequence and the purification domain may be useful for recovering the polypeptide.

15        Immunoassays.

CS198 polypeptides, including fragments, derivatives, and analogs thereof, or cells expressing such polypeptides, can be utilized in a variety of assays, many of which are described herein, for the detection of antibodies to GI tract tissue. They also can be used as immunogens to produce antibodies. These antibodies can be, 20 for example, polyclonal or monoclonal antibodies, chimeric, single chain and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

For example, antibodies generated against a polypeptide comprising a 25 sequence of the present invention can be obtained by direct injection of the polypeptide into an animal or by administering the polypeptide to an animal such as a mouse, rabbit, goat or human. A mouse, rabbit or goat is preferred. The polypeptide is selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof. The antibody so obtained then will bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide 30 can be used to generate antibodies that bind the native polypeptide. Such antibodies then can be used to isolate the polypeptide from test samples such as tissue suspected of containing that polypeptide. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures 35 can be used. Examples include the hybridoma technique as described by Kohler and Milstein, Nature 256:495-497 (1975), the trioma technique, the human B-cell

hybridoma technique as described by Kozbor et al, Immun. Today 4:72 (1983) and the EBV-hybridoma technique to produce human monoclonal antibodies as described by Cole et al., in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc, New York, NY, pp. 77-96 (1985). Techniques described for the 5 production of single chain antibodies can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. See, for example, U.S. Patent No. 4,946,778.

Various assay formats may utilize the antibodies of the present invention, including "sandwich" immunoassays and probe assays. For example, the antibodies 10 of the present invention, or fragments thereof, can be employed in various assay systems to determine the presence, if any, of CS198 antigen in a test sample. For example, in a first assay format, a polyclonal or monoclonal antibody or fragment thereof, or a combination of these antibodies, which has been coated on a solid phase, is contacted with a test sample, to form a first mixture. This first mixture is 15 incubated for a time and under conditions sufficient to form antigen/antibody complexes. Then, an indicator reagent comprising a monoclonal or a polyclonal antibody or a fragment thereof, or a combination of these antibodies, to which a signal generating compound has been attached, is contacted with the antigen/antibody complexes to form a second mixture. This second mixture then is 20 incubated for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence of CS198 antigen in the test sample and captured on the solid phase, if any, is determined by detecting the measurable signal generated by the signal generating compound. The amount of CS198 antigen present in the test sample is proportional to the signal generated.

25 In an alternative assay format, a mixture is formed by contacting: (1) a polyclonal antibody, monoclonal antibody, or fragment thereof, which specifically binds to CS198 antigen, or a combination of such antibodies bound to a solid support; (2) the test sample; and (3) an indicator reagent comprising a monoclonal antibody, polyclonal antibody, or fragment thereof, which specifically binds to a 30 different CS198 antigen (or a combination of these antibodies) to which a signal generating compound is attached. This mixture is incubated for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence, if any, of CS198 antigen present in the test sample and captured on the solid phase is determined by detecting the measurable signal generated by the signal generating 35 compound. The amount of CS198 antigen present in the test sample is proportional to the signal generated.

-47-

In another assay format, one or a combination of at least two monoclonal antibodies of the invention can be employed as a competitive probe for the detection of antibodies to CS198 antigen. For example, CS198 polypeptides such as the recombinant antigens disclosed herein, either alone or in combination, are coated on

5 a solid phase. A test sample suspected of containing antibody to CS198 antigen then is incubated with an indicator reagent comprising a signal generating compound and at least one monoclonal antibody of the invention for a time and under conditions sufficient to form antigen/antibody complexes of either the test sample and indicator reagent bound to the solid phase or the indicator reagent bound to the solid phase.

10 The reduction in binding of the monoclonal antibody to the solid phase can be quantitatively measured.

In yet another detection method, each of the monoclonal or polyclonal antibodies of the present invention can be employed in the detection of CS198 antigens in tissue sections, as well as in cells, by immunohistochemical analysis.

15 Cytochemical analysis wherein these antibodies are labeled directly (with, for example, fluorescein, colloidal gold, horseradish peroxidase, alkaline phosphatase, etc.) or are labeled by using secondary labeled anti-species antibodies (with various labels as exemplified herein) to track the histopathology of disease also are within the scope of the present invention.

20 In addition, these monoclonal antibodies can be bound to matrices similar to CNBr-activated Sepharose and used for the affinity purification of specific CS198 polypeptides from cell cultures or biological tissues such as to purify recombinant and native CS198 proteins.

The monoclonal antibodies of the invention also can be used for the 25 generation of chimeric antibodies for therapeutic use, or other similar applications.

The monoclonal antibodies or fragments thereof can be provided individually to detect CS198 antigens. Combinations of the monoclonal antibodies (and fragments thereof) provided herein also may be used together as components in a mixture or "cocktail" of at least one CS198 antibody of the invention, along with 30 antibodies which specifically bind to other CS198 regions, each antibody having different binding specificities. Thus, this cocktail can include the monoclonal antibodies of the invention which are directed to CS198 polypeptides disclosed herein and other monoclonal antibodies specific to other antigenic determinants of CS198 antigens or other related proteins.

35 The polyclonal antibody or fragment thereof which can be used in the assay formats should specifically bind to a CS198 polypeptide or other CS198

polypeptides additionally used in the assay. The polyclonal antibody used preferably is of mammalian origin such as, human, goat, rabbit or sheep polyclonal antibody which binds CS198 polypeptide. Most preferably, the polyclonal antibody is of rabbit origin. The polyclonal antibodies used in the assays can be used either 5 alone or as a cocktail of polyclonal antibodies. Since the cocktails used in the assay formats are comprised of either monoclonal antibodies or polyclonal antibodies having different binding specificity to CS198 polypeptides, they are useful for the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining the predisposition to, diseases and conditions of the GI tract, such as 10 GI tract cancer.

It is contemplated and within the scope of the present invention that CS198 antigen may be detectable in assays by use of a recombinant antigen as well as by use of a synthetic peptide or purified peptide, which peptide comprises an amino acid sequence of CS198. The amino acid sequence of such a polypeptide is selected 15 from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof. It also is within the scope of the present invention that different synthetic, recombinant or purified peptides, identifying different epitopes of CS198, can be used in combination in an assay for the detecting, diagnosing, staging, monitoring, prognosticating, preventing or treating, or determining the predisposition to diseases 20 and conditions of the GI tract, such as GI tract cancer. In this case, all of these peptides can be coated onto one solid phase; or each separate peptide may be coated onto separate solid phases, such as microparticles, and then combined to form a mixture of peptides which can be later used in assays. Furthermore, it is contemplated that multiple peptides which define epitopes from different antigens 25 may be used for the detection, diagnosis, staging, monitoring, prognosis, prevention or treatment of, or determining the predisposition to, diseases and conditions of the GI tract, such as GI tract cancer. Peptides coated on solid phases or labeled with detectable labels are then allowed to compete with those present in a patient sample (if any) for a limited amount of antibody. A reduction in binding of 30 the synthetic, recombinant, or purified peptides to the antibody (or antibodies) is an indication of the presence of CS198 antigen in the patient sample. The presence of CS198 antigen indicates the presence of GI tract tissue disease, especially GI tract cancer, in the patient. Variations of assay formats are known to those of ordinary skill in the art and many are discussed herein below.

35 In another assay format, the presence of anti-CS198 antibody and/or CS198 antigen can be detected in a simultaneous assay, as follows. A test sample is

-49-

simultaneously contacted with a capture reagent of a first analyte, wherein said capture reagent comprises a first binding member specific for a first analyte attached to a solid phase and a capture reagent for a second analyte, wherein said capture reagent comprises a first binding member for a second analyte attached to a second solid phase, to thereby form a mixture. This mixture is incubated for a time and under conditions sufficient to form capture reagent/first analyte and capture reagent/second analyte complexes. These so-formed complexes then are contacted with an indicator reagent comprising a member of a binding pair specific for the first analyte labeled with a signal generating compound and an indicator reagent comprising a member of a binding pair specific for the second analyte labeled with a signal generating compound to form a second mixture. This second mixture is incubated for a time and under conditions sufficient to form capture reagent/first analyte/indicator reagent complexes and capture reagent/second analyte/indicator reagent complexes. The presence of one or more analytes is determined by detecting a signal generated in connection with the complexes formed on either or both solid phases as an indication of the presence of one or more analytes in the test sample. In this assay format, recombinant antigens derived from the expression systems disclosed herein may be utilized, as well as monoclonal antibodies produced from the proteins derived from the expression systems as disclosed herein. For example, in this assay system, CS198 antigen can be the first analyte. Such assay systems are described in greater detail in EP Publication No. 0473065.

In yet other assay formats, the polypeptides disclosed herein may be utilized to detect the presence of antibody against CS198 antigen in test samples. For example, a test sample is incubated with a solid phase to which at least one polypeptide such as a recombinant protein or synthetic peptide has been attached. The polypeptide is selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof. These are reacted for a time and under conditions sufficient to form antigen/antibody complexes. Following incubation, the antigen/antibody complex is detected. Indicator reagents may be used to facilitate detection, depending upon the assay system chosen. In another assay format, a test sample is contacted with a solid phase to which a recombinant protein produced as described herein is attached, and also is contacted with a monoclonal or polyclonal antibody specific for the protein, which preferably has been labeled with an indicator reagent. After incubation for a time and under conditions sufficient for antibody/antigen complexes to form, the solid phase is separated from the free phase, and the label is detected in either the solid or free phase as an indication of the

-50-

presence of antibody against CS198 antigen. Other assay formats utilizing the recombinant antigens disclosed herein are contemplated. These include contacting a test sample with a solid phase to which at least one antigen from a first source has been attached, incubating the solid phase and test sample for a time and under

5 conditions sufficient to form antigen/antibody complexes, and then contacting the solid phase with a labeled antigen, which antigen is derived from a second source different from the first source. For example, a recombinant protein derived from a first source such as E. coli is used as a capture antigen on a solid phase, a test sample is added to the so-prepared solid phase, and following standard incubation

10 and washing steps as deemed or required, a recombinant protein derived from a different source (i.e., non-E. coli) is utilized as a part of an indicator reagent which subsequently is detected. Likewise, combinations of a recombinant antigen on a solid phase and synthetic peptide in the indicator phase also are possible. Any assay format which utilizes an antigen specific for CS198 produced or derived from a first

15 source as the capture antigen and an antigen specific for CS198 from a different second source is contemplated. Thus, various combinations of recombinant antigens, as well as the use of synthetic peptides, purified proteins and the like, are within the scope of this invention. Assays such as this and others are described in U.S. Patent No. 5,254,458.

20 Other embodiments which utilize various other solid phases also are contemplated and are within the scope of this invention. For example, ion capture procedures for immobilizing an immobilizable reaction complex with a negatively charged polymer (described in EP publication 0326100 and EP publication No. 0406473), can be employed according to the present invention to effect a fast

25 solution-phase immunochemical reaction. An immobilizable immune complex is separated from the rest of the reaction mixture by ionic interactions between the negatively charged poly-anion/immune complex and the previously treated, positively charged porous matrix and detected by using various signal generating systems previously described, including those described in chemiluminescent signal measurements as described in EPO Publication No. 0 273,115.

30 Also, the methods of the present invention can be adapted for use in systems which utilize microparticle technology including automated and semi-automated systems wherein the solid phase comprises a microparticle (magnetic or non-magnetic). Such systems include those described in, for example, published EPO

35 applications Nos. EP 0 425 633 and EP 0 424 634, respectively.

-51-

The use of scanning probe microscopy (SPM) for immunoassays also is a technology to which the monoclonal antibodies of the present invention are easily adaptable. In scanning probe microscopy, particularly in atomic force microscopy, the capture phase, for example, at least one of the monoclonal antibodies of the 5 invention, is adhered to a solid phase and a scanning probe microscope is utilized to detect antigen/antibody complexes which may be present on the surface of the solid phase. The use of scanning tunneling microscopy eliminates the need for labels which normally must be utilized in many immunoassay systems to detect 10 antigen/antibody complexes. The use of SPM to monitor specific binding reactions can occur in many ways. In one embodiment, one member of a specific binding partner (analyte specific substance which is the monoclonal antibody of the invention) is attached to a surface suitable for scanning. The attachment of the analyte specific substance may be by adsorption to a test piece which comprises a 15 solid phase of a plastic or metal surface, following methods known to those of ordinary skill in the art. Or, covalent attachment of a specific binding partner (analyte specific substance) to a test piece which test piece comprises a solid phase of derivatized plastic, metal, silicon, or glass may be utilized. Covalent attachment methods are known to those skilled in the art and include a variety of means to 20 irreversibly link specific binding partners to the test piece. If the test piece is silicon or glass, the surface must be activated prior to attaching the specific binding partner. Also, polyelectrolyte interactions may be used to immobilize a specific binding 25 partner on a surface of a test piece by using techniques and chemistries. The preferred method of attachment is by covalent means. Following attachment of a specific binding member, the surface may be further treated with materials such as serum, proteins, or other blocking agents to minimize non-specific binding. The 30 surface also may be scanned either at the site of manufacture or point of use to verify its suitability for assay purposes. The scanning process is not anticipated to alter the specific binding properties of the test piece.

While the present invention discloses the preference for the use of solid 35 phases, it is contemplated that the reagents such as antibodies, proteins and peptides of the present invention can be utilized in non-solid phase assay systems. These assay systems are known to those skilled in the art, and are considered to be within the scope of the present invention.

It is contemplated that the reagent employed for the assay can be provided in 35 the form of a test kit with one or more containers such as vials or bottles, with each container containing a separate reagent such as a probe, primer, monoclonal

antibody or a cocktail of monoclonal antibodies, or a polypeptide (e.g. recombinantly, synthetically produced or purified) employed in the assay. The polypeptide is selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof. Other components such as buffers, controls and the like, known to those of ordinary skill in art, may be included in such test kits. It also is contemplated to provide test kits which have means for collecting test samples comprising accessible body fluids, e.g., blood, urine, saliva and stool. Such tools useful for collection ("collection materials") include lancets and absorbent paper or cloth for collecting and stabilizing blood; swabs for collecting and stabilizing saliva; cups for collecting and stabilizing urine or stool samples. Collection materials, papers, cloths, swabs, cups and the like, may optionally be treated to avoid denaturation or irreversible adsorption of the sample. The collection materials also may be treated with or contain preservatives, stabilizers or antimicrobial agents to help maintain the integrity of the specimens. Test kits designed for the collection, stabilization and preservation of test specimens obtained by surgery or needle biopsy are also useful. It is contemplated that all kits may be configured in two components which can be provided separately; one component for collection and transport of the specimen and the other component for the analysis of the specimen. The collection component, for example, can be provided to the open market user while the components for analysis can be provided to others such as laboratory personnel for determination of the presence, absence or amount of analyte. Further, kits for the collection, stabilization and preservation of test specimens may be configured for use by untrained personnel and may be available in the open market for use at home with subsequent transportation to a laboratory for analysis of the test sample.

E. coli bacteria (clone 2055371) was deposited on 6/25/97 with the American Type Culture Collection (A.T.C.C.), 12301 Parklawn Drive, Rockville, Maryland 20852. The deposit was under the terms of the Budapest Treaty and will be maintained for a period of thirty (30) years from the date of deposit, or for five (5) years after the last request for the deposit, or for the enforceable period of the U.S. patent, whichever is longer. The deposit and any other deposited material described herein are provided for convenience only, and are not required to practice the present invention in view of the teachings provided herein. The cDNA sequence in all of the deposited material is incorporated herein by reference. Clone 2055371 was accorded A.T.C.C. Deposit No. 98462.

The present invention will now be described by way of examples, which are meant to illustrate, but not to limit, the scope of the present invention.

EXAMPLESExample 1: Identification of Gastrointestinal Tract Tissue Library CS198 Gene-Specific Clones

5        A. Library Comparison of Expressed Sequence Tags (ESTs) or Transcript Images. Partial sequences of cDNA clone inserts, so-called "expressed sequence tags" (ESTs), were derived from cDNA libraries made from GI tract tumor tissues, GI tract non-tumor tissues and numerous other tissues, both tumor and non-tumor and entered into a database (LIFESEQ™ database, available from Incyte

10      Pharmaceuticals, Palo Alto, CA) as gene transcript images. See International Publication No. WO 95/20681. (A transcript image is a listing of the number of EST's for each of the represented genes in a given tissue library. ESTs sharing regions of mutual sequence overlap are classified into clusters. A cluster is assigned a clone number from a representative 5' EST. Often, a cluster of interest can be

15      extended by comparing its consensus sequence with sequences of other EST's which did not meet the criteria for automated clustering. The alignment of all available clusters and single ESTs represent a contig from which a consensus sequence is derived.) The transcript images then were evaluated to identify EST sequences that were representative primarily of the GI tract tissue libraries. These

20      target clones then were ranked according to their abundance (occurrence) in the target libraries and their absence from background libraries. Higher abundance clones with low background occurrence were given higher study priority. ESTs corresponding to the consensus sequence of CS198 were found in 32.2% (19 of 59) of GI tract tissue libraries. ESTs corresponding to the consensus sequence

25      SEQUENCE ID NO 27, or fragments thereof, were found in 4.7% (27 of 573) of the other, non-GI tract, libraries of the data base. Therefore, the consensus sequence or fragment thereof was found more than 6 times more often in GI tract than non-GI tract tissues. Overlapping clones, 2682428 (SEQUENCE ID NO 7), 2682469 (SEQUENCE ID NO 8), 3359342 (SEQUENCE ID NO 9), 1736231

30      (SEQUENCE ID NO 10), 1734520 (SEQUENCE ID NO 11), 2596108 (SEQUENCE ID NO 12), 3388863 (SEQUENCE ID NO 13), g2322685 (SEQUENCE ID NO 14), 3988413 (SEQUENCE ID NO 15), 3615515 (SEQUENCE ID NO 16), 2055371 (SEQUENCE ID NO 17), 1431231 (SEQUENCE ID NO 18), 3253860 (SEQUENCE ID NO 19), 1753756

35      (SEQUENCE ID NO 20), 1887713 (SEQUENCE ID NO 21), 1803052 (SEQUENCE ID NO 22), 889029 (SEQUENCE ID NO 23), 2620906

-54-

(SEQUENCE ID NO 24), and 1754901 (SEQUENCE ID NO 25), were identified for further study. These represented the minimum number of clones that were needed to form the contig and from which, along with exon sequences derived from genomic clone g2804590 (SEQUENCE ID NOS 1-6), and the full-length sequence 5 of clone 2055371IH (SEQUENCE ID NO 26), the consensus sequence provided herein (SEQUENCE ID NO 27) was derived.

B. Generation of a Consensus Sequence. The nucleotide sequences of clones 2682428 (SEQUENCE ID NO 7), 2682469 (SEQUENCE ID NO 8), 3359342 (SEQUENCE ID NO 9), 1736231 (SEQUENCE ID NO 10), 1734520 10 (SEQUENCE ID NO 11), 2596108 (SEQUENCE ID NO 12), 3388863 (SEQUENCE ID NO 13), g2322685 (SEQUENCE ID NO 14), 3988413 (SEQUENCE ID NO 15), 3615515 (SEQUENCE ID NO 16), 2055371 (SEQUENCE ID NO 17), 1431231 (SEQUENCE ID NO 18), 3253860 (SEQUENCE ID NO 19), 1753756 (SEQUENCE ID NO 20), 1887713 15 (SEQUENCE ID NO 21), 1803052 (SEQUENCE ID NO 22), 889029 (SEQUENCE ID NO 23), 2620906 (SEQUENCE ID NO 24), 1754901 (SEQUENCE ID NO 25), 2055371IH (SEQUENCE ID NO 26), and the exon sequences derived from genomic clone g2804590 (SEQUENCE ID NOS 1-6), were entered in the Sequencher™ Program (available from Gene Codes Corporation, Ann 20 Arbor, MI) in order to generate a nucleotide alignment (contig map) and then generate their consensus sequence (SEQUENCE ID NO 27). Figures 1A-1G show the nucleotide sequence alignment of these clones and exon sequences, and their resultant nucleotide consensus sequence (SEQUENCE ID NO 27). Figure 2 presents the contig map depicting clones 2682428 (SEQUENCE ID NO 7), 2682469 (SEQUENCE ID NO 8), 3359342 (SEQUENCE ID NO 9), 1736231 25 (SEQUENCE ID NO 10), 1734520 (SEQUENCE ID NO 11), 2596108 (SEQUENCE ID NO 12), 3388863 (SEQUENCE ID NO 13), g2322685 (SEQUENCE ID NO 14), 3988413 (SEQUENCE ID NO 15), 3615515 (SEQUENCE ID NO 16), 2055371 (SEQUENCE ID NO 17), 1431231 30 (SEQUENCE ID NO 18), 3253860 (SEQUENCE ID NO 19), 1753756 (SEQUENCE ID NO 20), 1887713 (SEQUENCE ID NO 21), 1803052 (SEQUENCE ID NO 22), 889029 (SEQUENCE ID NO 23), 2620906 (SEQUENCE ID NO 24), 1754901 (SEQUENCE ID NO 25), 2055371IH 35 (SEQUENCE ID NO 26), and the exon sequences derived from genomic clone g2804590 (SEQUENCE ID NOS 1-6) which form overlapping regions of the CS198 gene, and the resultant consensus nucleotide sequence (SEQUENCE ID NO

-55-

27) of these clones in a graphic display. A possible G/T polymorphism was noted at position 163 in the consensus nucleotide sequence (SEQUENCE ID NO 27). The ratio of G's to T's noted in the LIFESEQ™ database at position 163 was 3:1. Following generation of the consensus sequence, a three-frame translation was 5 performed on SEQUENCE ID NO 27. The second forward frame was found to have an open reading frame encoding a 679 residue amino acid sequence which is presented as SEQUENCE ID NO 42. A 215 residue amino acid sequence representing the C-terminal portion of this molecule is presented as SEQUENCE ID NO 47. The 679 residue polypeptide sequence depicted in SEQUENCE ID NO 42 10 has substantial sequence identity with the conceptual translation of a "hypothetical human protein of unknown function," deposited with GenBank on January 23, 1998 under Accession No. AC004030. The polypeptide sequence of SEQUENCE ID NO 42 was also compared with published sequences using software and techniques known to those skilled in the art. The polypeptide sequence of a protein 15 similar to Plasmodium falciparum glutamic acid-rich precursor was found to be partially homologous to that of the CS198 polypeptide of SEQUENCE ID NO 42. The sequence for this partially homologous protein is deposited with GenBank under Accession No. D87440.

20 Example 2: Sequencing of CS198 EST-Specific Clones

The DNA sequence of clone 2055371 of the CS198 gene contig was determined (SEQUENCE ID NO 26) using dideoxy termination sequencing with dye terminators following known methods [F. Sanger et al., PNAS U.S.A. 74:5463 (1977)]. This full-length sequence is referred to herein as clone 2055371IH 25 (SEQUENCE ID NO 26).

Because the pINCY vector (available from Incyte Pharmaceuticals, Inc., Palo Alto, CA) contains universal priming sites just adjacent to the 3' and 5' ligation junctions of the inserts, approximately 300 bases of the insert were sequenced in both directions using universal primers (SEQUENCE ID NO 30 and SEQUENCE 30 ID NO 31, available from New England Biolabs, Beverly, MA, and Applied Biosystems Inc, Foster City, CA). The sequencing reactions were run on a polyacrylamide denaturing gel, and the sequences were determined by an Applied Biosystems 377 Sequencer (available from Applied Biosystems, Foster City, CA). Additional sequencing primers (SEQUENCE ID NOS 32-39) were designed from 35 sequence information of the consensus sequence, SEQUENCE ID NO 27. These

primers then were used to determine the remaining DNA sequence of the cloned insert from each DNA strand, as previously described.

Example 3: Nucleic Acid

5        A. RNA Extraction from Tissue. Total RNA was isolated from GI tract tissues and from non-GI tract tissues. Various methods were utilized, including but not limited to the lithium chloride/urea technique, known in the art and described by Kato et al. (J. Virol. 61:2182-2191, 1987), and TRIzol™ (Gibco-BRL, Grand Island, NY).

10        Briefly, tissue was placed in a sterile conical tube on ice and 10-15 volumes of 3 M LiCl, 6 M urea, 5 mM EDTA, 0.1 M  $\beta$ -mercaptoethanol, 50 mM Tris-HCl (pH 7.5) were added. The tissue was homogenized with a Polytron® homogenizer (Brinkman Instruments, Inc., Westbury, NY) for 30-50 sec on ice. The solution was transferred to a 15 ml plastic centrifuge tube and placed overnight at -20°C. The 15 tube was centrifuged for 90 minutes at 9,000 x g at 0-4°C and the supernatant was immediately decanted. Ten ml of 3 M LiCl were added and the tube was vortexed for 5 sec. The tube was centrifuged for 45 minutes at 11,000 x g at 0-4°C. The decanting, resuspension in LiCl, and centrifugation was repeated and the final pellet was air dried and suspended in 2 ml of 1 mM EDTA, 0.5% SDS, 10 mM Tris (pH 20 7.5). Twenty microliters (20  $\mu$ l) of Proteinase K (20 mg/ml) were added, and the solution was incubated for 30 minutes at 37°C with occasional mixing. One-tenth volume (0.22-0.25 ml) of 3 M NaCl was added and the solution was vortexed before transfer into another tube containing 2 ml of phenol/chloroform/isoamyl alcohol (PCI). The tube was vortexed for 1-3 sec and centrifuged for 20 minutes at 25 3,000 x g at 10°C. The PCI extraction was repeated and followed by two similar extractions with chloroform/isoamyl alcohol (CI). The final aqueous solution was transferred to a prechilled 15 ml Corex glass tube containing 6 ml of absolute ethanol, the tube was covered with parafilm, and placed at -20°C overnight. The tube was centrifuged for 30 minutes at 10,000 x g at 0-4°C and the ethanol 30 supernatant was decanted immediately. The RNA pellet was washed four times with 10 ml of 75% ice-cold ethanol and the final pellet was air dried for 15 minutes at room temperature. The RNA was suspended in 0.5 ml of 10 mM TE (pH 7.6, 1 mM EDTA) and its concentration was determined spectrophotometrically. RNA samples were aliquoted and stored at -70°C as ethanol precipitates.

35        The quality of the RNA was determined by agarose gel electrophoresis (see Example 5, Northern Blot Analysis) and staining with 0.5  $\mu$ g/ml ethidium bromide

for one hour. RNA samples that did not contain intact rRNAs were excluded from the study.

Alternatively, for RT-PCR analysis, 1 ml of Ultraspec RNA reagent was added to 120 mg of pulverized tissue in a 2.0 ml polypropylene microfuge tube, 5 homogenized with a Polytron<sup>®</sup> homogenizer (Brinkman Instruments, Inc., Westbury, NY) for 50 sec and placed on ice for 5 minutes. Then, 0.2 ml of chloroform was added to each sample, followed by vortexing for 15 sec. The sample was placed on ice for another 5 min, followed by centrifugation at 12,000 x g for 15 minutes at 4°C. The upper layer was collected and transferred to another 10 RNase-free 2.0 ml microfuge tube. An equal volume of isopropanol was added to each sample, and the solution was placed on ice for 10 minutes. The sample was centrifuged at 12,000 x g for 10 minutes at 4°C, and the supernatant was discarded. The remaining pellet was washed twice with cold 75% ethanol, resuspended by vortexing, and the resuspended material was then pelleted by centrifugation at 7500 15 x g for 5 minutes at 4°C. Finally, the RNA pellet was dried in a Speedvac (Savant, Farmingdale, NY) for 5 minutes and reconstituted in RNase-free water.

**B. RNA Extraction from Blood Mononuclear Cells.** Mononuclear cells are isolated from blood samples from patients by centrifugation using Ficoll-Hypaque as follows. A 10 ml volume of whole blood is mixed with an equal volume of RPMI 20 Medium (Gibco-BRL, Grand Island, NY). This mixture is then underlaid with 10 ml of Ficoll-Hypaque (Pharmacia, Piscataway, NJ) and centrifuged for 30 minutes at 200 x g. The buffy coat containing the mononuclear cells is removed, diluted to 50 ml with Dulbecco's PBS (Gibco-BRL, Grand Island, NY) and the mixture centrifuged for 10 minutes at 200 x g. After two washes, the resulting pellet is 25 resuspended in Dulbecco's PBS to a final volume of 1 ml.

RNA is prepared from the isolated mononuclear cells as described by N. Kato et al., *J. Virology* 61: 2182-2191 (1987). Briefly, the pelleted mononuclear cells are brought to a final volume of 1 ml and then are resuspended in 250 µL of PBS and mixed with 2.5 ml of 3 M LiCl, 6 M urea, 5 mM EDTA, 0.1 M 2-30 mercaptoethanol, 50 mM Tris-HCl (pH 7.5). The resulting mixture is homogenized and incubated at -20°C overnight. The homogenate is centrifuged at 8,000 RPM in a Beckman J2-21M rotor for 90 minutes at 0-4°C. The pellet is resuspended in 10 ml. of 3 M LiCl by vortexing and then centrifuged at 10,000 RPM in a Beckman J2-35 21M rotor centrifuge for 45 minutes at 0-4°C. The resuspending and pelleting steps then are repeated. The pellet is resuspended in 2 ml of 1 mM EDTA, 0.5% SDS, 10 mM Tris (pH 7.5) and 400 µg Proteinase K with vortexing and then it is incubated

at 37°C for 30 minutes with shaking. One tenth volume of 3 M NaCl then is added and the mixture is vortexed. Proteins are removed by two cycles of extraction with phenol/ chloroform/ isoamyl alcohol (PCI) followed by one extraction with chloroform/ isoamyl alcohol (CI). RNA is precipitated by the addition of 6 ml of 5 absolute ethanol followed by overnight incubation at -20°C. After the precipitated RNA is collected by centrifugation, the pellet is washed 4 times in 75% ethanol. The pelleted RNA is then dissolved in solution containing 1 mM EDTA, 10 mM Tris-HCl (pH 7.5).

Non-GI tract tissues are used as negative controls. The mRNA can be 10 further purified from total RNA by using commercially available kits such as oligo dT cellulose spin columns (RediCol™ from Pharmacia, Uppsala, Sweden) for the isolation of poly-adenylated RNA. Total RNA or mRNA can be dissolved in lysis buffer (5 M guanidine thiocyanate, 0.1 M EDTA, pH 7.0) for analysis in the ribonuclease protection assay.

15 C. RNA Extraction from polysomes. Tissue is minced in saline at 4°C and mixed with 2.5 volumes of 0.8 M sucrose in a TK<sub>150</sub>M (150 mM KCl, 5 mM MgCl<sub>2</sub>, 50 mM Tris-HCl, pH 7.4) solution containing 6 mM 2-mercaptoethanol. The tissue is homogenized in a Teflon-glass Potter homogenizer with five strokes at 100-200 rpm followed by six strokes in a Dounce homogenizer, as described by B. 20 Mechler, Methods in Enzymology 152:241-248 (1987). The homogenate then is centrifuged at 12,000 x g for 15 minutes at 4°C to sediment the nuclei. The polysomes are isolated by mixing 2 ml of the supernatant with 6 ml of 2.5 M sucrose in TK<sub>150</sub>M and layering this mixture over 4 ml of 2.5 M sucrose in TK<sub>150</sub>M in a 38 ml polyallomer tube. Two additional sucrose TK<sub>150</sub>M solutions are 25 successively layered onto the extract fraction; a first layer of 13 ml 2.05 M sucrose followed by a second layer of 6 ml of 1.3 M sucrose. The polysomes are isolated by centrifuging the gradient at 90,000 x g for 5 hr at 4°C. The fraction then is taken from the 1.3 M sucrose/2.05 M sucrose interface with a siliconized pasteur pipette and diluted in an equal volume of TE (10 mM Tris-HCl, pH 7.4, 1 mM EDTA). An 30 equal volume of 90°C SDS buffer (1% SDS, 200 mM NaCl, 20 mM Tris-HCl, pH 7.4) is added and the solution is incubated in a boiling water bath for 2 minutes. Proteins next are digested with a Proteinase-K digestion (50 mg/ml) for 15 minutes at 37°C. The mRNA is purified with 3 equal volumes of phenol-chloroform 35 extractions followed by precipitation with 0.1 volume of 2 M sodium acetate (pH 5.2) and 2 volumes of 100% ethanol at -20°C overnight. The precipitated RNA is recovered by centrifugation at 12,000 x g for 10 minutes at 4°C. The RNA is dried

and resuspended in TE (pH 7.4) or distilled water. The resuspended RNA then can be used in a slot blot or dot blot hybridization assay to check for the presence of CS198 mRNA (see Example 6).

5 The quality of nucleic acid and proteins is dependent on the method of preparation used. Each sample may require a different preparation technique to maximize isolation efficiency of the target molecule. These preparation techniques are within the skill of the ordinary artisan.

#### Example 4: Ribonuclease Protection Assay

10 A. Synthesis of Labeled Complementary RNA (cRNA) Hybridization Probe and Unlabeled Sense Strand. Labeled antisense and unlabeled sense riboprobes are transcribed from the CS198 gene cDNA sequence which contains a 5' RNA polymerase promoter such as SP6 or T7. The sequence may be from a vector containing the appropriate CS198 cDNA insert, or from a PCR-generated product of 15 the insert using PCR primers which incorporate a 5' RNA polymerase promoter sequence. For example, the described plasmid, clone 2055371 or another comparable clone, containing the CS198 gene cDNA sequence, flanked by opposed SP6 and T7 polymerase or other RNA polymerase promoters, is purified using a Qiagen Plasmid Purification Kit (Qiagen, Chatsworth, CA). Then 10 µg of the 20 plasmid DNA are linearized by cutting with Dde I restriction enzyme for 1 hr at 37°C. The linearized plasmid DNA is purified using the QIAprep kit (Qiagen, Chatsworth, CA) and used for the synthesis of antisense transcript from the appropriate promoter using the Riboprobe® in vitro Transcription System (Promega Corporation, Madison, WI), as described by the supplier's instructions, 25 incorporating either 6.3 µM (alpha<sup>32</sup>P) UTP (Amersham Life Sciences, Inc. Arlington Heights, IL) or 100-500 µM biotinylated UTP as a label. To generate the sense strand, 10 µg of the purified plasmid DNA are cut with restriction enzymes XbaI and NotI, and transcribed as above from the appropriate SP6 or T7 promoter. Both sense and antisense strands are isolated by spin column chromatography. 30 Unlabeled sense strand is quantitated by UV absorption at 260 nm.

35 B. Hybridization of Labeled Probe to Target. Frozen tissue is pulverized to powder under liquid nitrogen and 100-500 mg are dissolved in 1 ml of lysis buffer, available as a component of the Direct Protect™ Lysate RNase Protection kit (Ambion, Inc., Austin, TX). Further dissolution can be achieved using a tissue homogenizer. In addition, a dilution series of a known amount of sense strand in mouse liver lysate is made for use as a positive control. Finally, 45 µl of solubilized

-60-

tissue or diluted sense strand is mixed directly with either (1)  $1 \times 10^5$  cpm of radioactively labeled probe, or (2) 250 pg of non-isotopically labeled probe in 5  $\mu$ l of lysis buffer. Hybridization is allowed to proceed overnight at 37°C. See, T. Kaabache et al., *Anal. Biochem.* 232:225-230 (1995).

5 **C. RNase Digestion.** RNA that is not hybridized to probe is removed from the reaction as per the Direct Protect™ protocol using a solution of RNase A and RNase T1 for 30 minutes at 37°C, followed by removal of RNase by Proteinase-K digestion in the presence of sodium sarcosyl. Hybridized fragments protected from digestion are then precipitated by the addition of an equal volume of isopropanol and 10 placed at -70°C for 3 hr. The precipitates are collected by centrifugation at 12,000 x g for 20 minutes.

15 **D. Fragment Analysis.** The precipitates are dissolved in denaturing gel loading dye (80% formamide, 10 mM EDTA (pH 8.0), 1 mg/ml xylene cyanol, 1 mg/ml bromophenol blue), heat denatured, and electrophoresed in 6% polyacrylamide TBE, 8 M urea denaturing gels. The gels are imaged and analyzed using the STORM™ storage phosphor autoradiography system (Molecular Dynamics, Sunnyvale, CA). Quantitation of protected fragment bands, expressed in 20 femtograms (fg), is achieved by comparing the peak areas obtained from the test samples to those from the known dilutions of the positive control sense strand (see Section B, *supra*). The results are expressed in molecules of CS198 RNA/cell and as a image rating score. In cases where non-isotopic labels are used, hybrids are transferred from the gels to membranes (nylon or nitrocellulose) by blotting and then analyzed using detection systems that employ streptavidin alkaline phosphatase conjugates and chemiluminescence or chemifluorescence reagents.

25 Detection of a product comprising a sequence selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof, is indicative of the presence of CS198 mRNA(s), suggesting a diagnosis of a GI tract tissue disease or condition, such as GI tract cancer.

30 **Example 5: Northern Blotting**

The Northern blot technique was used to identify a specific size RNA species in a complex population of RNA using agarose gel electrophoresis and nucleic acid hybridization. Briefly, 5-10  $\mu$ g of total RNA (see Example 3, Nucleic Acid Preparation) were incubated in 15  $\mu$ l of a solution containing 40 mM morpholinopropanesulfonic acid (MOPS) (pH 7.0), 10 mM sodium acetate, 1 mM EDTA, 2.2 M formaldehyde, 50% v/v formamide for 15 minutes at 65°C. The

denatured RNA was mixed with 2  $\mu$ l of loading buffer (50% glycerol, 1 mM EDTA, 0.4% bromophenol blue, 0.4% xylene cyanol) and loaded into a denaturing 1.0% agarose gel containing 40 mM MOPS (pH 7.0), 10 mM sodium acetate, 1 mM EDTA and 2.2 M formaldehyde. The gel was electrophoresed at 60 V for 1.5 hr, 5 stained with 0.5  $\mu$ g/ml ethidium bromide for one hour and rinsed in RNase free water for 30-45 minutes. RNA was transferred from the gel onto nylon membranes (Brightstar-Plus, Ambion, Inc., Austin, TX) for 1.5 hours using the downward alkaline capillary transfer method (Chomczynski, *Anal. Biochem.* 201:134-139, 1992). The filter was rinsed with 1X SSC and RNA was crosslinked to the filter 10 using a Stratalinker (Stratagene, Inc., La Jolla, CA) on the autocrosslinking mode and dried for 15 minutes. The membrane was then placed into a hybridization tube containing 20 ml of preheated prehybridization solution (5X SSC, 50% formamide, 5X Denhardt's solution, 100  $\mu$ g/ml denatured salmon sperm DNA) and incubated in a 42°C hybridization oven for at least 3 hr. While the blot was prehybridizing, a 15  $^{32}$ P-labeled random-primed probe was generated using the CS198 insert according to the manufacturer's instructions (Gibco-BRL, Grand Island, NY). Half of the probe was boiled for 10 min, quick chilled on ice and added to the hybridization tube. Hybridization was carried out at 42°C for at least 12 hr. The hybridization solution was discarded and the filter was washed twice in 30 ml of 3X SSC, 0.1% SDS at 20 42°C for 15 min, followed by two washes in 30 ml of 0.3X SSC, 0.1% SDS at 60°C for 15 minutes each. The filter was wrapped in Saran Wrap and exposed to Kodak XAR-Omat film for 8-120 hr and the film was developed for analysis.

The results of the analysis of CS198 hybridization to a Northern blot containing GI tract and non-GI tract tissues are shown in Figure 3 which depicts an 25 ethidium bromide (EtBr)-stained RNA gel and the CS198 Northern blot. The positions of RNA size standards (in Kb) are shown to the left of each panel. Figure 3 shows that the CS198 probe detected an approximately 3.0 Kb RNA in the colon specimen (lane 3), more weakly detected the same size band in the prostate specimen (lane 10), and weakly detected a product of about 5 Kb in the kidney specimen (lane 30 4). No hybridization was observed in any of the other nine non-GI tract RNA samples.

Detection of a product comprising a sequence selected from the group 35 consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof, is indicative of the presence of CS198 mRNA(s), suggesting a diagnosis of a GI tract tissue disease or condition, such as GI tract cancer.

Example 6: Dot Blot/Slot Blot

Dot and slot blot assays are quick methods to evaluate the presence of a specific nucleic acid sequence in a complex mix of nucleic acid. To perform such assays, up to 50 µg of RNA are mixed in 50 µl of 50% formamide, 7% 5 formaldehyde, 1X SSC, incubated 15 minutes at 68°C, and then cooled on ice. Then, 100 µl of 20X SSC are added to the RNA mixture and loaded under vacuum onto a manifold apparatus that has a prepared nitrocellulose or nylon membrane. The membrane is soaked in water, 20X SSC for 1 hour, placed on two sheets of 10 20X SSC prewet Whatman #3 filter paper, and loaded into a slot blot or dot blot vacuum manifold apparatus. The slot blot is analyzed with probes prepared and labeled as described in Example 4, supra. Detection of mRNA corresponding to a sequence selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof, is an indication of the presence of CS198, suggesting a diagnosis of a GI tract tissue disease or condition, such as GI tract 15 cancer.

Other methods and buffers which can be utilized in the methods described in Examples 5 and 6, but not specifically detailed herein, are known in the art and are described in J. Sambrook et al., supra.

20 Detection of a product comprising a sequence selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof, is indicative of the presence of CS198 mRNA(s), suggesting a diagnosis of a GI tract tissue disease or condition, such as GI tract cancer.

Example 7: In Situ Hybridization

25 This method is useful to directly detect specific target nucleic acid sequences in cells using detectable nucleic acid hybridization probes.

Tissues are prepared with cross-linking fixative agents such as 30 paraformaldehyde or glutaraldehyde for maximum cellular RNA retention. See, L. Angerer et al., Methods in Cell Biol. 35:37-71 (1991). Briefly, the tissue is placed in greater than 5 volumes of 1% glutaraldehyde in 50 mM sodium phosphate, pH 7.5 at 4°C for 30 minutes. The solution is changed with fresh glutaraldehyde 35 solution (1% glutaraldehyde in 50 mM sodium phosphate, pH 7.5) for a further 30 minutes fixing. The fixing solution should have an osmolality of approximately 0.375% NaCl. The tissue is washed once in isotonic NaCl to remove the phosphate.

-63-

The fixed tissues then are embedded in paraffin as follows. The tissue is dehydrated through a series of increasing ethanol concentrations for 15 minutes each: 50% (twice), 70% (twice), 85%, 90% and then 100% (twice). Next, the tissue is soaked in two changes of xylene for 20 minutes each at room temperature. The 5 tissue is then soaked in two changes of a 1:1 mixture of xylene and paraffin for 20 minutes each at 60°C; and then in three final changes of paraffin for 15 minutes each.

Next, the tissue is cut in 5  $\mu$ m sections using a standard microtome and placed on a slide previously treated with a tissue adhesive such as 3-aminopropyltriethoxysilane.

10 Paraffin is removed from the tissue by two 10 minutes xylene soaks and rehydrated in a series of decreasing ethanol concentrations: 99% (twice), 95%, 85%, 70%, 50%, 30%, and then distilled water (twice). The sections are pre-treated with 0.2 M HCl for 10 minutes and permeabilized with 2  $\mu$ g/ml Proteinase-K at 37°C for 15 minutes.

15 Labeled Riboprobes transcribed from the CS198 gene plasmid (see Example 4) are hybridized to the prepared tissue sections and incubated overnight at 56°C in 3X standard saline extract and 50% formamide. Excess probe is removed by washing in 2X standard saline citrate and 50% formamide followed by digestion with 100  $\mu$ g/ml RNase A at 37°C for 30 minutes. Fluorescence probe is visualized 20 by illumination with ultraviolet (UV) light under a microscope. Fluorescence in the cytoplasm is indicative of CS198 mRNA. Alternatively, the sections can be visualized by autoradiography.

25 Detection of a product comprising a sequence selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof, is indicative of the presence of CS198 mRNA(s), suggesting a diagnosis of a GI tract tissue disease or condition, such as GI tract cancer.

#### Example 8: Reverse Transcription PCR

A. One Step RT-PCR Assay. Target-specific primers are designed to detect 30 the above-described target sequences by reverse transcription PCR using methods known in the art. One step RT-PCR is a sequential procedure that performs both RT and PCR in a single reaction mixture. The procedure is performed in a 200  $\mu$ l reaction mixture containing 50 mM (N,N,-bis[2-Hydroxyethyl]glycine), pH 8.15, 81.7 mM KOAc, 33.33 mM KOH, 0.01 mg/ml bovine serum albumin, 0.1 mM 35 ethylene diaminetetraacetic acid, 0.02 mg/ml NaN<sub>3</sub>, 8% w/v glycerol, 150  $\mu$ M each of dNTP, 0.25  $\mu$ M each primer, 5U rTth polymerase, 3.25 mM Mn(OAc)<sub>2</sub>, and 5  $\mu$ l

of target RNA (see Example 3). Since RNA and the rTth polymerase enzyme are unstable in the presence of Mn(OAc)<sub>2</sub>, the Mn(OAc)<sub>2</sub> should be added just before target addition. Optimal conditions for cDNA synthesis and thermal cycling readily can be determined by those skilled in the art. The reaction is incubated in a Perkin-  
5 Elmer Thermal Cycler 480. Optimal conditions for cDNA synthesis and thermal cycling can readily be determined by those skilled in the art. Conditions which may be found useful include cDNA synthesis at 60°-70°C for 15-45 minutes and 30-45 amplification cycles at 94°C, 1 min; 55°-70°C, 1 min; 72°C, 2 minutes. One step RT-PCR also may be performed by using a dual enzyme procedure with Taq  
10 polymerase and a reverse transcriptase enzyme, such as MMLV or AMV RT enzymes.

B. Traditional RT-PCR. A traditional two-step RT-PCR reaction was performed, as described by K.Q. Hu et al., *Virology* 181:721-726 (1991). Briefly, 1.0 µg of extracted mRNA (see Example 3) was reverse transcribed in a 20 µl  
15 reaction mixture containing 1X PCR II buffer (Perkin-Elmer), 5 mM MgCl<sub>2</sub>, 1 mM dNTP, 20 U RNasin, 2.5 µM random hexamers, and 50 U MMLV (Moloney murine leukemia virus) reverse transcriptase (RT). Reverse transcription was performed at 42°C for 30 minutes in a PE-480 thermal cycler, followed by further incubation at 95°C for 5 minutes to inactivate the RT. PCR was performed using 2  
20 µl of the cDNA reaction in a final PCR reaction volume of 50 µl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 µM dNTP, 0.4 µM of each sense and antisense primer, SEQUENCE ID NO 40 and SEQUENCE ID NO 41, respectively, and 2.5 U of Taq polymerase. The reaction was incubated in an MJ Research Model PTC-200 as follows: Denaturation at 94°C for 2 minutes followed  
25 by 35 cycles of amplification (94°C, 45 sec; 59°C, 45 sec; 72°C, 2 minutes); a final extension (72°C, 5 min); and a soak at 4°C.

C. PCR Fragment Analysis. The correct products were verified by size determination using gel electrophoresis and staining with SYBR® Green nucleic acid gel stain (Molecular Probes, Eugene, OR). After the gel was stained with SYBR®  
30 Green nucleic acid gel stain in TBE buffer for 30 minutes, it was visualized using the Molecular Dynamics Storm imaging system. In Figure 4, lane 1 shows a 100 bp MW marker set. Lane 2 is a placental DNA negative control. The other lanes are as follows: (lane 3) normal colon; (lane 4) colon cancer; (lanes 5-6) breast cancer; (lane 7) benign prostatic hyperplasia; (lane 8) prostate cancer; (lane 9) benign prostatic  
35 hyperplasia; (lanes 10-11) normal lung; and (lane 12) lung cancer. The expected 496 bp RT-PCR amplicon was detected not only in the colon specimens (lanes 3-4),

but also in a prostate cancer specimen (lane 8), a normal lung specimen (lane 10), and in a lung cancer specimen (lane 12). The band was not detected in the placental DNA control.

In Figure 5, lane 1 shows a 100 bp MW marker set. Lane 2 is a placental DNA negative control. The other lanes are as follows: (lanes 3 and 6) normal colon; and (lanes 4, 5, and 7) colon cancer. The expected 496 bp RT-PCR amplicon was detected in all 5 colon tissue specimens (lanes 3-7) but not in the placental DNA control (lane 2).

Detection of a product comprising a sequence selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof, is indicative of the presence of CS198 mRNA(s), suggesting a diagnosis of a GI tract tissue disease or condition, such as GI tract cancer.

#### Example 9: OH-PCR

15        **A. Probe selection and Labeling.** Target-specific primers and probes are designed to detect the above-described target sequences by oligonucleotide hybridization PCR. International Publication Nos WO 92/10505, published 25 June 1992, and WO 92/11388, published 9 July 1992, teach methods for labeling oligonucleotides at their 5' and 3' ends, respectively. According to one known 20 method for labeling an oligonucleotide, a label-phosphoramidite reagent is prepared and used to add the label to the oligonucleotide during its synthesis. For example, see N. T. Thuong et al., *Tet. Letters* 29(46):5905-5908 (1988); or J. S. Cohen et al., published U.S. Patent Application 07/246,688 (NTIS ORDER No. PAT-APPL-7-246,688) (1989). Preferably, probes are labeled at their 3' end to prevent 25 participation in PCR and the formation of undesired extension products. For one step OH-PCR, the probe should have a  $T_m$  at least 15°C below the  $T_m$  of the primers. The primers and probes are utilized as specific binding members, with or without detectable labels, using standard phosphoramidite chemistry and/or post-synthetic labeling methods which are well-known to one skilled in the art.

30        **B. One Step Oligo Hybridization PCR.** OH-PCR is performed on a 200  $\mu$ l reaction containing 50 mM (N,N,-bis[2-Hydroxyethyl]glycine), pH 8.15, 81.7 mM KOAc, 33.33 mM KOH, 0.01 mg/ml bovine serum albumin, 0.1 mM ethylene diaminetetraacetic acid, 0.02 mg/ml NaN<sub>3</sub>, 8% w/v glycerol, 150  $\mu$ M each of dNTP, 0.25  $\mu$ M each primer, 3.75 nM probe, 5U rTth polymerase, 3.25 mM Mn(OAc)<sub>2</sub>, 35 and 5  $\mu$ l blood equivalents of target (see Example 3). Since RNA and the rTth polymerase enzyme are unstable in the presence of Mn(OAc)<sub>2</sub>, the Mn(OAc)<sub>2</sub> should

be added just before target addition. The reaction is incubated in a Perkin-Elmer Thermal Cycler 480. Optimal conditions for cDNA synthesis and thermal cycling can be readily determined by those skilled in the art. Conditions which may be found useful include cDNA synthesis (60°C, 30 min), 30-45 amplification cycles (94°C, 40 sec; 55-70°C, 60 sec), oligo-hybridization (97°C, 5 min; 15°C, 5 min; 15°C soak). The correct reaction product contains at least one of the strands of the PCR product and an internally hybridized probe.

5           C. OH-PCR Product Analysis. Amplified reaction products are detected on an LCx® analyzer system (available from Abbott Laboratories, Abbott Park, IL).

10          Briefly, the correct reaction product is captured by an antibody labeled microparticle at a capturable site on either the PCR product strand or the hybridization probe, and the complex is detected by binding of a detectable antibody conjugate to either a detectable site on the probe or the PCR strand. Only a complex containing a PCR strand hybridized with the internal probe is detectable. The detection of this complex

15          then is indicative of the presence of CS198 mRNA, suggesting a diagnosis of a GI tract disease or condition, such as GI tract cancer.

Many other detection formats exist which can be used and/or modified by those skilled in the art to detect the presence of amplified or non-amplified CS198-derived nucleic acid sequences including, but not limited to, ligase chain reaction (LCR, Abbott Laboratories, Abbott Park, IL); Q-beta replicase (Gene-Trak™, Naperville, Illinois), branched chain reaction (Chiron, Emeryville, CA) and strand displacement assays (Becton Dickinson, Research Triangle Park, NC).

#### Example 10: Synthetic Peptide Production

25          Synthetic peptides were modeled and then prepared based upon the predicted amino acid sequence of the CS198 polypeptide consensus sequence (see Example 1). In particular, a number of CS198 peptides derived from SEQUENCE ID NO 42 were prepared, including the peptides of SEQUENCE ID NO 43, SEQUENCE ID NO 44, SEQUENCE ID NO 45, and SEQUENCE ID NO 46. All peptides were

30          synthesized on a Symphony Peptide Synthesizer (available from Rainin Instrument Co, Emeryville, CA) using Fmoc chemistry, standard cycles and in-situ HBTU activation. Cleavage and deprotection conditions were as follows: a volume of 2.5 ml of cleavage reagent (77.5% v/v trifluoroacetic acid, 15% v/v ethanedithiol, 2.5% v/v water, 5% v/v thioanisole, 1-2% w/v phenol) were added to the resin, and

35          agitated at room temperature for 2-4 hours. Then the filtrate was removed and the peptide was precipitated from the cleavage reagent with cold diethyl ether. Each

peptide was filtered, purified via reverse-phase preparative HPLC using a water/acetonitrile/0.1% TFA gradient, and lyophilized. The product was confirmed by mass spectrometry.

The purified peptides were used to immunize animals (see Example 14).

5

Example 11a: Expression of Protein in a Cell Line Using Plasmid 577

A. Construction of a CS198 Expression Plasmid. Plasmid 577, described in U.S. patent application Serial No. 08/478,073, filed June 7, 1995, has been constructed for the expression of secreted antigens in a permanent cell line. This 10 plasmid contains the following DNA segments: (a) a 2.3 Kb fragment of pBR322 containing bacterial beta-lactamase and origin of DNA replication; (b) a 1.8 Kb cassette directing expression of a neomycin resistance gene under control of HSV-1 thymidine kinase promoter and poly-A addition signals; (c) a 1.9 Kb cassette directing expression of a dihydrofolate reductase gene under the control of a SV-40 15 (Simian Virus 40) promoter and poly-A addition signals; (d) a 3.5 Kb cassette directing expression of a rabbit immunoglobulin heavy chain signal sequence fused to a modified hepatitis C virus (HCV) E2 protein under the control of the SV40 T-Ag promoter and transcription enhancer, the hepatitis B virus surface antigen (HBsAg) enhancer I followed by a fragment of Herpes Simplex Virus-1 (HSV-1) 20 genome providing poly-A addition signals; and (e) a residual 0.7 Kb fragment of SV40 genome late region of no function in this plasmid. All of the segments of the vector were assembled by standard methods known to those skilled in the art of molecular biology.

Plasmids for the expression of secretable CS198 proteins are constructed by 25 replacing the hepatitis C virus E2 protein coding sequence in plasmid 577 with that of a CS198 polynucleotide sequence selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof, as follows. Digestion of plasmid 577 with XbaI releases the hepatitis C virus E2 gene fragment. The resulting plasmid backbone allows insertion of the CS198 cDNA insert 30 downstream of the rabbit immunoglobulin heavy chain signal sequence which directs the expressed proteins into the secretory pathway of the cell. The CS198 cDNA fragment is generated by PCR using standard procedures. Encoded in the sense PCR primer sequence is an XbaI site, immediately followed by a 12 nucleotide sequence that encodes the amino acid sequence Ser-Asn-Glu-Leu 35 ("SNEL") to promote signal protease processing, efficient secretion and final product stability in culture fluids. Immediately following this 12 nucleotide

sequence the primer contains nucleotides complementary to template sequences encoding amino acids of the CS198 gene. The antisense primer incorporates a sequence encoding the following eight amino acids just before the stop codons: Asp-Tyr-Lys-Asp-Asp-Asp-Lys (SEQUENCE ID NO 48). Within this sequence is 5 incorporated a recognition site to aid in analysis and purification of the CS198 protein product. A recognition site (termed "FLAG") that is recognized by a commercially available monoclonal antibody designated anti-FLAG M2 (Eastman Kodak, Co., New Haven, CT) can be utilized, as well as other comparable sequences and their corresponding antibodies. For example, PCR is performed 10 using GeneAmp® reagents obtained from Perkin-Elmer-Cetus, as directed by the supplier's instructions. PCR primers are used at a final concentration of 0.5 µM. PCR is performed on the CS198 plasmid template in a 100 µl reaction for 35 cycles (94°C, 30 seconds; 55°C, 30 seconds; 72°C, 90 seconds) followed by an extension cycle of 72°C for 10 minutes.

15        B. Transfection of Dihydrofolate Reductase Deficient Chinese Hamster Ovary Cells. The plasmid described, supra, is transfected into CHO/dhfr- cells (DXB-111, Uriacio et al., PNAS 77:4451-4466 (1980)). These cells are available from the A.T.C.C., 12301 Parklawn Drive, Rockville, MD 20852, under Accession No. CRL 9096. Transfection is carried out using the cationic liposome-mediated 20 procedure described by P. L. Felgner et al., PNAS 84:7413-7417 (1987). Particularly, CHO/dhfr- cells are cultured in Ham's F-12 media supplemented with 10% fetal calf serum, L-glutamine (1 mM) and freshly seeded into a flask at a density of 5 - 8 x 10<sup>5</sup> cells per flask. The cells are grown to a confluence of between 25 60 and 80% for transfection. Twenty micrograms (20 µg) of plasmid DNA is added to 1.5 ml of Opti-MEM I medium and 100 µl of Lipofectin Reagent (Gibco-BRL; Grand Island, NY) are added to a second 1.5 ml portion of Opti-MEM I media. The two solutions are mixed and incubated at room temperature for 20 minutes. After the culture medium is removed from the cells, the cells are rinsed 3 times with 5 ml of Opti-MEM I medium. The Opti-MEM I-Lipofection-plasmid DNA solution then 30 is overlaid onto the cells. The cells are incubated for 3 hr at 37°C, after which time the Opti-MEM I-Lipofectin-DNA solution is replaced with culture medium for an additional 24 hr prior to selection.

35        C. Selection and Amplification. One day after transfection, cells are passaged 1:3 and incubated with dhfr/G418 selection medium (hereafter, "F-12 minus medium G"). Selection medium is Ham's F-12 with L-glutamine and without hypoxanthine, thymidine and glycine (JRH Biosciences, Lenexa, Kansas) and 300

μg per ml G418 (Gibco-BRL; Grand Island, NY). Media volume-to-surface area ratios of 5 ml per 25 cm<sup>2</sup> are maintained. After approximately two weeks, DHFR/G418 cells are expanded to allow passage and continuous maintenance in F-12 minus medium G.

5        Amplification of each of the transfected CS198 cDNA sequences is achieved by stepwise selection of DHFR<sup>+</sup>, G418<sup>+</sup> cells with methotrexate [reviewed by R. Schimke, *Cell* 37:705-713 (1984)]. Cells are incubated with F-12 minus medium G containing 150 nM methotrexate (MTX) (Sigma, St. Louis, MO) for approximately two weeks until resistant colonies appear. Further gene amplification is achieved by  
10      selection of 150 nM adapted cells with 5 μM MTX.

15      D. Antigen Production. F-12 minus medium G supplemented with 5 μM MTX is overlaid onto just confluent monolayers for 12 to 24 hr at 37°C in 5% CO<sub>2</sub>. The growth medium is removed and the cells are rinsed 3 times with Dulbecco's phosphate buffered saline (PBS) (with calcium and magnesium) (Gibco-BRL, Grand Island, NY) to remove the remaining media/serum which may be present. Cells then are incubated with VAS custom medium (VAS custom formulation with L-glutamine with HEPES without phenol red, available from JRH Bioscience; Lenexa, KS, product number 52-08678P), for 1 hr at 37°C in 5% CO<sub>2</sub>. Cells then are overlaid with VAS for production at 5 ml per T flask. Medium is removed after  
20      seven days of incubation, retained, and then frozen to await purification with harvests 2, 3 and 4. The monolayers are overlaid with VAS for 3 more seven day harvests.

25      E. Analysis of GI tract Tissue Gene CS198 Antigen Expression. Aliquots of VAS supernatants from the cells expressing the CS198 protein construct are analyzed, either by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using standard methods and reagents known in the art (Laemmli discontinuous gels), or by mass spectrometry.

30      F. Purification. Purification of the CS198 protein containing the FLAG sequence is performed by immunoaffinity chromatography using an affinity matrix comprising anti-FLAG M2 monoclonal antibody covalently attached to agarose by hydrazide linkage (Eastman Kodak Co., New Haven, CT). Prior to affinity purification, protein in pooled VAS medium harvests from roller bottles is exchanged into 50 mM Tris-HCl (pH 7.5), 150 mM NaCl buffer using a Sephadex G-25 (Pharmacia Biotech Inc., Uppsala, Sweden) column. Protein in this buffer is applied to the anti-FLAG M2 antibody affinity column. Non-binding protein is eluted by washing the column with 50 mM Tris-HCl (pH 7.5), 150 mM NaCl

-70-

buffer. Bound protein is eluted using an excess of FLAG peptide in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl. The excess FLAG peptide can be removed from the purified CS198 protein by gel electrophoresis or HPLC.

5 Although plasmid 577 is utilized in this example, it is known to those skilled in the art that other comparable expression systems, such as CMV, can be utilized herein with appropriate modifications in reagent and/or techniques and are within the skill of the ordinary artisan.

10 The largest cloned insert containing the coding region of the CS198 gene is then sub-cloned into either (i) a eukaryotic expression vector which may contain, for example, a cytomegalovirus (CMV) promoter and/or protein fusible sequences which aid in protein expression and detection, or (ii) a bacterial expression vector containing a superoxide-dismutase (SOD) and CMP-KDO synthetase (CKS) or other protein fusion gene for expression of the protein sequence. Methods and vectors which are useful for the production of polypeptides which contain fusion 15 sequences of SOD are described in EPO 0196056, published October 1, 1986, and those containing fusion sequences of CKS are described in EPO Publication No. 0331961, published September 13, 1989. This so-purified protein can be used in a variety of techniques, including, but not limited to animal immunization studies, solid phase immunoassays, etc.

20

Example 11b: Expression of Protein in a Cell Line Using pcDNA3.1/Myc-His

A. Construction of a CS198 Expression Plasmid. Plasmid pcDNA3.1/Myc-His (Cat. # V855-20, Invitrogen, Carlsbad, CA) has been constructed, in the past, for the expression of secreted antigens by most mammalian cell lines. Expressed 25 protein inserts are fused to a myc-his peptide tag. The myc-his tag is a 21 residue amino acid sequence having the following sequence: Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu-Asn-Met-His-Thr-Glu-His-His-His-His-His (SEQUENCE ID NO 49) and comprises a myc epitope and a polyhistidine sequence which are useful for the purification of an expressed fusion protein using either anti-myc or anti-his 30 affinity columns, or metalloprotein binding columns.

Plasmids for the expression of secretable CS198 proteins are constructed by inserting an CS198 polynucleotide sequence selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof. Prior to construction of an CS198 expression plasmid, the CS198 cDNA sequence is first 35 cloned into a pCR<sup>®</sup>-Blunt vector as follows.

The CS198 cDNA fragment is generated by PCR using standard procedures. For example, PCR is performed using Stratagene<sup>®</sup> reagents obtained from Stratagene, La Jolla, CA, as directed by the supplier's instructions. PCR primers are used at a final concentration of 0.5  $\mu$ M. PCR using 5 U of pfu polymerase 5 (Stratagene) is performed on the CS198 plasmid template (see Example 2) in a 50  $\mu$ l reaction for 30 cycles (94°C, 1 min; 65°C, 1.5 min; 72°C, 3 min) followed by an extension cycle at 72°C for 8 min. The sense PCR primer sequence comprises nucleotides which are either complementary to the pINCY vector directly upstream of the CS198 gene insert or which incorporate a 5' EcoRI restriction site, an adjacent 10 downstream protein translation consensus initiator, and a 3' nucleic acid sequence which is the same sense as the 5'-most end of the CS198 cDNA insert. The antisense primer incorporates a 5' NotI restriction sequence and a sequence complementary to the 3' end of the CS198 cDNA insert just upstream of the 3'-most, in-frame stop codon. Five microliters (5  $\mu$ l) of the resulting blunt-ended PCR 15 product are ligated into 25 ng of linearized pCR<sup>®</sup>-Blunt vector (Invitrogen, Carlsbad, CA) interrupting the lethal ccdB gene of the vector. The resulting ligated vector is transfected into TOP10 E. coli (Invitrogen, Carlsbad, CA) using a One Shot<sup>™</sup> transformation kit (Invitrogen, Carlsbad, CA) following the supplier's directions. The transfected cells are grown on LB-Kan (50  $\mu$ g/ml kanamycin) selection plates at 20 37°C. Only cells containing a plasmid with an interrupted ccdB gene will grow after transfection (Grant, S.G.N., PNAS USA 87:4645-4649 (1990)). Transfected colonies are picked and grown up in 3 ml of LB-Kan broth at 37°C. Plasmid DNA is isolated using a QIAprep<sup>®</sup> (Qiagen Inc., Santa Clarita, CA) procedure, as directed by the supplier's instructions. The DNA is cut with EcoRI or SnaBI, and NotI 25 restriction enzymes to release the CS198 insert fragment. The fragment is run on 1% Seakem<sup>®</sup> LE agarose/0.5  $\mu$ g/ml ethidium bromide/TE gel, visualized by UV irradiation, excised and purified using QIAquick<sup>™</sup> (Qiagen Inc., Santa Clarita, CA) procedures, as directed by the supplier's instructions.

The pcDNA3.1/Myc-His plasmid DNA is linearized by digestion with EcoRI 30 or SnaBI, and NotI in the polylinker region of the plasmid DNA. The resulting plasmid DNA backbone allows insertion of the CS198 purified cDNA fragment, supra, downstream of a CMV promoter which directs expression of the proteins in mammalian cells. The ligated plasmid is transfected into DH5 alpha<sup>™</sup> cells (GibcoBRL, Gaithersburg, MD) as directed by the supplier's instructions. Briefly, 35 10 ng of pcDNA3.1/Myc-His containing an CS198 insert is added to 50  $\mu$ l of competent DH5 alpha cells, and the contents are mixed gently. The mixture is

incubated on ice for 30 min, heat shocked for 20 sec at 37°C, and placed on ice for an additional 2 min. Upon addition of 0.95 ml of LB medium, the mixture is incubated for 1 h at 37°C while shaking at 225 rpm. The transfected cells are then plated onto 100 mm LB/Amp (50 µg/ml ampicillin) plates and grown at 37°C.

5 Colonies are picked and grown in 3 ml of LB/Amp broth. Plasmid DNA is purified using a QIAprep® kit. Presence of the insert is confirmed using techniques known to those skilled in the art including, but not limited to, restriction digestion and gel analysis. See, e.g., J. Sambrook et al., supra.

**B. Transfection of Human Embryonic Kidney 293 Cells.** The CS198 expression plasmid described supra is purified from the DH5 alpha cells using a QIAfilter™ Maxi kit (Qiagen, Chatsworth, CA), and then transfected into HEK293 cells (F.L. Graham et al., J. Gen. Vir. 36:59-72 (1977)). These cells are available from the A.T.C.C., 12301 Parklawn Drive, Rockville, MD 20852, under Accession No. CRL 1573. Transfection is carried out using the cationic lipofectamine-mediated procedure described by P. Hawley-Nelson et al., Focus 15:73 (1993). Particularly, HEK293 cells are cultured in 10 ml DMEM media supplemented with 10% fetal bovine serum (FBS), L-glutamine (2 mM) and freshly seeded into 100 mm culture plates at a density of  $9 \times 10^6$  cells per plate. The cells are grown at 37°C to a confluence of between 70% and 80% for transfection. Eight micrograms (8 µg) of plasmid DNA is added to 800 µl of Opti-MEM I® medium (Gibco-BRL, Grand Island, NY), and 48-96 µl of Lipofectamine™ Reagent (Gibco-BRL, Grand Island, NY) is added to a second 800 µl portion of Opti-MEM I® media. The two solutions are mixed and incubated at room temperature for 15-30 min. After the culture medium is removed from the cells, the cells are washed once with 10 ml of serum-free DMEM. The Opti-MEM I®-Lipofectamine-plasmid DNA solution is diluted in 6.4 ml of serum-free DMEM and then overlaid onto the cells. The cells are incubated for 5 h at 37°C, after which time, an additional 8 ml of DMEM with 20% FBS is added. After 18-24 h, the old medium is aspirated, and the cells are overlaid with 5 ml of fresh DMEM with 10% FBS. Supernatants and cell extracts are analyzed for CS198 gene activity 72 h after transfection.

**C. Analysis of GI Tract Tissue Gene CS198 Antigen Expression.** The culture supernatant, supra, is transferred to cryotubes and stored on ice. HEK293 cells are harvested by washing twice with 10 ml cold Dulbecco's PBS and lysing by addition of 1.5 ml of CAT lysis buffer (Boehringer Mannheim, Indianapolis, IN), followed by incubation for 30 min at room temperature. Lysate is transferred to 1.7 ml polypropylene microfuge tubes and centrifuged at 1000 x g for 10 min. The

Tween<sup>®</sup> 20. The prepared microtiter plate can then be used to screen polyclonal antisera for the presence of CS198 antibodies (see Example 17).

Although pcDNA3.1/Myc-His is utilized in this example, it is known to those skilled in the art that other comparable expression systems can be utilized 5 herein with appropriate modifications in reagent and/or techniques and are within the skill of one of ordinary skill in the art. The largest cloned insert containing the coding region of the CS198 gene is sub-cloned into either (i) a eukaryotic expression vector which may contain, for example, a cytomegalovirus (CMV) promoter and/or protein fusible sequences which aid in protein expression and detection, or (ii) a 10 bacterial expression vector containing a superoxide-dismutase (SOD) and CMP-KDO synthetase (CKS) or other protein fusion gene for expression of the protein sequence. Methods and vectors which are useful for the production of polypeptides which contain fusion sequences of SOD are described in European patent application No. EP 0 196 056, published October 1, 1986, and vectors containing fusion 15 sequences of CKS are described in European patent application No. EP 0 331 961, published September 13, 1989. The purified protein can be used in a variety of techniques, including but not limited to, animal immunization studies, solid phase immunoassays, etc.

20 Example 12: Chemical Analysis of GI tract Tissue Proteins

A. Analysis of Tryptic Peptide Fragments Using MS. Sera from patients with GI tract disease, such as GI tract cancer, sera from patients with no GI tract disease, extracts of GI tract tissues or cells from patients with GI tract disease, such as GI tract cancer, extracts of GI tract tissues or cells from patients with no GI tract 25 disease, and extracts of tissues or cells from other non-diseased or diseased organs of patients, are run on a polyacrylamide gel using standard procedures and stained with Coomassie Blue. Sections of the gel suspected of containing the unknown polypeptide are excised and subjected to an in-gel reduction, acetamidation and tryptic digestion. P. Jeno et al, Anal. Bio. 224:451-455 (1995) and J. Rosenfeld et 30 al, Anal. Bio. 203:173-179 (1992). The gel sections are washed with 100 mM NH<sub>4</sub>HCO<sub>3</sub> and acetonitrile. The shrunken gel pieces are swollen in digestion buffer (50 mM NH<sub>4</sub>HCO<sub>3</sub>, 5 mM CaCl<sub>2</sub> and 12.5 µg/ml trypsin) at 4°C for 45 minutes. The supernatant is aspirated and replaced with 5 to 10 µl of digestion buffer without 35 trypsin and allowed to incubate overnight at 37°C. Peptides are extracted with 3 changes of 5% formic acid and acetonitrile and evaporated to dryness. The peptides are adsorbed to approximately 0.1 µl of POROS R2 sorbent (Perseptive

supernatant is transferred to new cryotubes and stored on ice. Aliquots of cell supernatants and the lysate of the cells expressing the CS198 protein construct are analyzed for the presence of CS198 recombinant protein. The aliquots can be analyzed using SDS-polyacrylamide gel electrophoresis (SDS-PAGE), using 5 standard methods and reagents known in the art. See, e.g., J. Sambrook et al., supra. The gels can then be blotted onto a solid medium such as nitrocellulose, nytran, or the like, and the CS198 protein band can be visualized using western blotting techniques with anti-myc epitope or anti-histidine monoclonal antibodies (Invitrogen, Carlsbad, CA) or CS198 polyclonal serum (see Example 14). 10 Alternatively, the expressed CS198 recombinant protein can be analyzed by mass spectrometry (see Example 12).

**D. Purification.** Purification of the CS198 recombinant protein containing the myc-his sequence is performed using the Xpress<sup>®</sup> affinity chromatography system (Invitrogen, Carlsbad, CA) containing a nickel-charged agarose resin which 15 specifically binds polyhistidine residues. Supernatants from 10 x 100 mm plates, prepared as described supra, are pooled and passed over the nickel-charged column. Non-binding protein is eluted by washing the column with 50 mM Tris-HCl (pH 7.5)/150 mM NaCl buffer, leaving only the myc-his fusion proteins. Bound CS198 recombinant protein then is eluted from the column using either an excess of 20 imidazole or histidine, or a low pH buffer. Alternatively, the recombinant protein can also be purified by binding at the myc-his sequence to an affinity column consisting of either anti-myc or anti-histidine monoclonal antibodies conjugated through a hydrazide or other linkage to an agarose resin and eluting with an excess of myc peptide or histidine, respectively.

25 The purified recombinant protein can then be covalently cross-linked to a solid phase, such as N-hydroxysuccinimide-activated sepharose columns (Pharmacia Biotech, Piscataway, NJ), as directed by supplier's instructions. These columns containing covalently linked CS198 recombinant protein, can then be used to purify anti-CS198 antibodies from rabbit or mouse sera (see Examples 13 and 30 14).

**E. Coating Microtiter Plates with CS198 Expressed Proteins.** Supernatant from a 100 mm plate, as described supra, is diluted in an appropriate volume of PBS. 100  $\mu$ l of the resulting mixture is placed into each well of a Reacti-Bind<sup>TM</sup> metal chelate microtiter plate (Pierce, Rockford, IL), incubated at room temperature 35 while shaking, and followed by three washes with 200  $\mu$ l each of PBS with 0.05%

Biosystems, Framingham, Massachusetts) trapped in the tip of a drawn gas chromatography capillary tube by dissolving them in 10  $\mu$ l of 5% formic acid and passing it through the capillary. The adsorbed peptides are washed with water and eluted with 5% formic acid in 60% methanol. The eluant is passed directly into the 5 spraying capillary of an API III mass spectrometer (Perkin-Elmer Sciex, Thornhill, Ontario, Canada) for analysis by nano-electrospray mass spectrometry. M. Wilm et al., Int. J. Mass Spectrom. Ion Process 136:167-180 (1994) and M. Wilm et al., Anal. Chem. 66:1-8 (1994). The masses of the tryptic peptides are determined from the mass spectrum obtained off the first quadrupole. Masses corresponding to 10 predicted peptides can be further analyzed in MS/MS mode to give the amino acid sequence of the peptide.

B. Peptide Fragment Analysis Using LC/MS. The presence of polypeptides predicted from mRNA sequences found in hyperplastic disease tissues also can be confirmed using liquid chromatography/tandem mass spectrometry (LC/MS/MS). 15 D. Hess et al., METHODS, A Companion to Methods in Enzymology 6:227-238 (1994). The serum specimen or tumor extract from the patient is denatured with SDS and reduced with dithiothreitol (1.5 mg/ml) for 30 minutes at 90°C followed by alkylation with iodoacetamide (4 mg/ml) for 15 minutes at 25°C. Following acrylamide electrophoresis, the polypeptides are electroblotted to a cationic 20 membrane and stained with Coomassie Blue. Following staining, the membranes are washed and sections thought to contain the unknown polypeptides are cut out and dissected into small pieces. The membranes are placed in 500  $\mu$ l microcentrifuge tubes and immersed in 10 to 20  $\mu$ l of proteolytic digestion buffer (100 mM Tris-HCl, pH 8.2, containing 0.1 M NaCl, 10% acetonitrile, 2 mM CaCl<sub>2</sub>, 25 and 5  $\mu$ g/ml trypsin) (Sigma, St. Louis, MO). After 15 hr at 37°C, 3  $\mu$ l of saturated urea and 1  $\mu$ l of 100  $\mu$ g/ml trypsin are added and incubated for an additional 5 hr at 37°C. The digestion mixture is acidified with 3  $\mu$ l of 10% trifluoroacetic acid and centrifuged to separate supernatant from membrane. The supernatant is injected directly onto a microbore, reverse phase HPLC column and eluted with a linear 30 gradient of acetonitrile in 0.05% trifluoroacetic acid. The eluate is fed directly into an electrospray mass spectrometer, after passing through a stream splitter if necessary to adjust the volume of material. The data is analyzed following the procedures set forth in Example 12, Section A.

Example 13: Gene Immunization Protocol

A. In Vivo Antigen Expression. Gene immunization circumvents protein purification steps by directly expressing an antigen *in vivo* after inoculation of the appropriate expression vector. Also, production of antigen by this method may 5 allow correct protein folding and glycosylation since the protein is produced in mammalian tissue. The method utilizes insertion of the gene sequence into a plasmid which contains a CMV promoter, expansion and purification of the plasmid and injection of the plasmid DNA into the muscle tissue of an animal. Preferred animals include mice and rabbits. See, for example, H. Davis et al., Human Molecular  
10 Genetics 2:1847-1851 (1993). After one or two booster immunizations, the animal can then be bled, ascites fluid collected, or the animal's spleen can be harvested for production of hybridomas.

B. Plasmid Preparation and Purification. CS198 cDNA sequences are generated from the CS198 cDNA-containing vector using appropriate PCR primers 15 containing suitable 5' restriction sites following the procedures described in Example 11. The PCR product is cut with appropriate restriction enzymes and inserted into a vector which contains the CMV promoter (for example, pRc/CMV or pcDNA3 vectors from Invitrogen, San Diego, CA). This plasmid then is expanded in the appropriate bacterial strain and purified from the cell lysate using a CsCl gradient or 20 a Qiagen plasmid DNA purification column. All these techniques are familiar to one of ordinary skill in the art of molecular biology.

C. Immunization Protocol. Anesthetized animals are immunized intramuscularly with 0.1-100 µg of the purified plasmid diluted in PBS or other DNA uptake enhancers (Cardiotoxin, 25% sucrose). See, for 25 example, H. Davis et al., Human Gene Therapy 4:733-740 (1993); and P. W. Wolff et al., Biotechniques 11:474-485 (1991). One to two booster injections are given at monthly intervals.

D. Testing and Use of Antiserum. Animals are bled and the resultant sera tested for antibody using peptides synthesized from the known 30 gene sequence (see Example 16) using techniques known in the art, such as western blotting or EIA techniques. Antisera produced by this method can then be used to detect the presence of the antigen in a patient's tissue or cell extract, or in a patient's serum, by ELISA or Western blotting techniques, such as those described in Examples 15 through 18.

Example 14: Production of Antibodies Against CS198

**A. Production of Polyclonal Antisera.** Antiserum against CS198 was prepared by injecting rabbits with peptides whose sequences were derived from that of the predicted amino acid sequence of the CS198 consensus nucleotide sequence (SEQUENCE ID NO 42). The synthesis of these peptides (SEQUENCE ID NO 43, SEQUENCE ID NO 44, SEQUENCE ID NO 45, and SEQUENCE ID NO 46) is described in Example 10. Peptides used as immunogens were not conjugated to a carrier such as keyhole limpet hemocyanine, KLH, (i.e., they were unconjugated.).

Animal Immunization. Female white New Zealand rabbits weighing 10 2 kg or more were used for raising polyclonal antiserum. One animal was immunized per unconjugated peptide (SEQUENCE ID NO 43, SEQUENCE ID NO 44, SEQUENCE ID NO 45, or SEQUENCE ID NO 46). One week prior to the first immunization, blood samples (5 to 10 ml) were obtained from the animals to serve as a non-immune prebleed sample.

15 Unconjugated peptides, SEQUENCE ID NO 43, SEQUENCE ID NO 44, SEQUENCE ID NO 45, and SEQUENCE ID NO 46, were used to prepare the primary immunogen by emulsifying 0.5 ml of the peptide at a concentration of 2 mg/ml in PBS (pH 7.2) which contained 0.5 ml of complete Freund's adjuvant (CFA) (Difco, Detroit, MI). The immunogen was injected into several sites of the 20 animal via subcutaneous, intraperitoneal, and intramuscular routes of administration. Four weeks following the primary immunization, a booster immunization was administered. The immunogen used for the booster immunization dose was prepared by emulsifying 0.5 ml of the same unconjugated peptide used for the primary immunogen, except that the peptide now was diluted to 1 mg/ml with 0.5 ml 25 of incomplete Freund's adjuvant (IFA) (Difco, Detroit, MI). Again, the booster dose was administered into several sites via subcutaneous, intraperitoneal and intramuscular types of injections. The animals were bled (5 ml) two weeks after the booster immunizations and each serum was tested for immunoreactivity to the peptide as described below. The booster and bleed schedule was repeated at 4 week 30 intervals until an adequate titer was obtained. The titer or concentration of antiserum was determined by using unconjugated peptides in a microtiter EIA as described in Example 17, below. An antibody titer of 1:500 or greater was considered an adequate titer for further use and study.

Table 1. Titer of rabbit anti-CS198 peptide antisera (13 week bleed)

Peptide	Immunogen	Titer
SEQ. ID NO 43		> 62,500
SEQ. ID NO 44		58,000
SEQ. ID NO 45		7,200
SEQ. ID NO 46		10,500

B. Production of Monoclonal Antibody.

5        1. Immunization Protocol. Mice are immunized using peptides which can either be conjugated to a carrier such as KLH [prepared as described hereinbelow, or unconjugated (i.e., not conjugated to a carrier such as KLH)] except that the amount of the unconjugated or conjugated peptide for monoclonal antibody production in mice is one-tenth the amount used to produce polyclonal antisera in  
10      rabbits. Thus, the primary immunogen consists of 100 µg of unconjugated or conjugated peptide in 0.1 ml of CFA emulsion while the immunogen used for booster immunizations consists of 50 µg of unconjugated or conjugated peptide in 0.1 ml of IFA. Hybridomas for the generation of monoclonal antibodies are prepared and screened using standard techniques. The methods used for  
15      monoclonal antibody development follow procedures known in the art such as those detailed in Kohler and Milstein, Nature 256:494 (1975) and reviewed in J.G.R. Hurrel, ed., Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press, Inc., Boca Raton, FL (1982). Another method of monoclonal antibody development which is based on the Kohler and Milstein method is that of L.T.  
20      Mimms et al., Virology 176:604-619 (1990).

25        The immunization regimen (per mouse) consists of a primary immunization with additional booster immunizations. The primary immunogen used for the primary immunization consists of 100 µg of unconjugated or conjugated peptide in 50 µl of PBS (pH 7.2) previously emulsified in 50 µl of CFA. Booster immunizations performed at approximately two weeks and four weeks post primary immunization consist of 50 µg of unconjugated or conjugated peptide in 50 µl of PBS (pH 7.2) emulsified with 50 µl IFA. A total of 100 µl of this immunogen are inoculated intraperitoneally and subcutaneously into each mouse. Individual mice are screened for immune response by microtiter plate enzyme immunoassay (EIA) as  
30      described in Example 17 approximately four weeks after the third immunization.

Mice are inoculated either intravenously, intrasplenically or intraperitoneally with 50  $\mu$ g of unconjugated or conjugated peptide in PBS (pH 7.2) approximately fifteen weeks after the third immunization..

Three days after this intravenous boost, splenocytes are fused with, 5 for example, Sp2/0-Ag14 myeloma cells (Milstein Laboratories, England) using the polyethylene glycol (PEG) method. The fusions are cultured in Iscove's Modified Dulbecco's Medium (IMDM) containing 10% fetal calf serum (FCS), plus 1% hypoxanthine, aminopterin and thymidine (HAT). Bulk cultures are screened by 10 microtiter plate EIA following the protocol in Example 17. Clones reactive with the peptide used as an immunogen and non-reactive with other peptides (i.e., peptides of CS198 not used as the immunogen) are selected for final expansion. Clones thus selected are expanded, aliquoted and frozen in IMDM containing 10% FCS and 10% dimethyl sulfoxide, (DMSO).

2. Peptide Conjugation. Peptide is conjugated to maleimide 15 activated KLH (commercially available as Imject<sup>®</sup>, available from Pierce Chemical Company, Rockford, IL). Imject<sup>®</sup> contains about 250 moles of reactive maleimide groups per mole of hemocyanine. The activated KLH is dissolved in phosphate buffered saline (PBS, pH 8.4) at a concentration of about 7.7 mg/ml. The peptide is conjugated through cysteines occurring in the peptide sequence, or to a cysteine 20 previously added to the synthesized peptide in order to provide a point of attachment. The peptide is dissolved in DMSO (Sigma Chemical Company, St. Louis, MO) and reacted with the activated KLH at a mole ratio of about 1.5 moles of peptide per mole of reactive maleimide attached to the KLH. A procedure for the 25 conjugation of peptide is provided hereinbelow. It is known to the ordinary artisan that the amounts, times and conditions of such a procedure can be varied to optimize peptide conjugation.

The conjugation reaction described hereinbelow is based on obtaining 3 mg of KLH peptide conjugate ("conjugated peptide"), which contains about 0.77  $\mu$ moles of reactive maleimide groups. This quantity of peptide conjugate usually is 30 adequate for one primary injection and four booster injections for production of polyclonal antisera in a rabbit. Briefly, peptide is dissolved in DMSO at a concentration of 1.16  $\mu$ moles/100  $\mu$ l of DMSO. One hundred microliters (100  $\mu$ l) of the DMSO solution are added to 380  $\mu$ l of the activated KLH solution prepared as described hereinabove, and 20  $\mu$ l of PBS (pH 8.4) are added to bring the volume to 35 500  $\mu$ l. The reaction is incubated overnight at room temperature with stirring. The extent of reaction is determined by measuring the amount of unreacted thiol in the

reaction mixture. The difference between the starting concentration of thiol and the final concentration is assumed to be the concentration of peptide which has coupled to the activated KLH. The amount of remaining thiol is measured using Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid), Pierce Chemical Company, Rockford, IL). Cysteine standards are made at a concentration of 0, 0.1, 0.5, 2, 5 and 20 mM by dissolving 35 mg of cysteine HCl (Pierce Chemical Company, Rockford, IL) in 10 ml of PBS (pH 7.2) and diluting the stock solution to the desired concentration(s). The photometric determination of the concentration of thiol is accomplished by placing 200  $\mu$ l of PBS (pH 8.4) in each well of an Immulon 2<sup>®</sup> microwell plate (Dynex Technologies, Chantilly, VA). Next, 10  $\mu$ l of standard or reaction mixture are added to each well. Finally, 20  $\mu$ l of Ellman's reagent at a concentration of 1 mg/ml in PBS (pH 8.4) are added to each well. The wells are incubated for 10 minutes at room temperature, and the absorbance of all wells is read at 415 nm with a microplate reader (such as the BioRad Model 3550, BioRad, Richmond, CA). The absorbance of the standards is used to construct a standard curve and the thiol concentration of the reaction mixture is determined from the standard curve. A decrease in the concentration of free thiol is indicative of a successful conjugation reaction. Unreacted peptide is removed by dialysis against PBS (pH 7.2) at room temperature for 6 hours. The conjugate is stored at 2-8°C if it is to be used immediately; otherwise, it is stored at -20°C or colder.

3. Production of Ascites Fluid Containing Monoclonal Antibodies. Frozen hybridoma cells prepared as described hereinabove are thawed and placed into expansion culture. Viable hybridoma cells are inoculated intraperitoneally into Pristane treated mice. Ascitic fluid is removed from the mice, pooled, filtered through a 0.2  $\mu$  filter and subjected column required for the purification.

4. Purification of Monoclonal Antibodies From Ascites Fluid. Briefly, filtered and thawed to an immunoglobulin class G (IgG) analysis to determine the volume of the Protein A ascites fluid is mixed with an equal volume of Protein A sepharose binding buffer (1.5 M glycine, 3.0 M NaCl, pH 8.9) and refiltered through a 0.2  $\mu$  filter. The volume of the Protein A column is determined by the quantity of IgG present in the ascites fluid. The eluate then is dialyzed against PBS (pH 7.2) overnight at 2-8°C. The dialyzed monoclonal antibody is sterile filtered and dispensed in aliquots. The immunoreactivity of the purified monoclonal antibody is confirmed by determining its ability to specifically bind to the peptide used as the immunogen by use of the EIA microtiter plate assay procedure of Example 17. The specificity of the purified monoclonal antibody is confirmed by

determining its lack of binding to irrelevant peptides such as peptides of CS198 not used as the immunogen. The purified anti-CS198 monoclonal thus prepared and characterized is placed at either 2-8°C for short term storage or at -80°C for long term storage.

5        5. Further Characterization of Monoclonal Antibody. The isotype and subtype of the monoclonal antibody produced as described hereinabove can be determined using commercially available kits (available from Amersham, Inc., Arlington Heights, IL). Stability testing also can be performed on the monoclonal antibody by placing an aliquot of the monoclonal antibody in continuous storage at  
10      2-8°C and assaying optical density (OD) readings throughout the course of a given period of time.

15        C. Use of Recombinant Proteins as Immunogens. It is within the scope of the present invention that recombinant proteins made as described herein can be utilized as immunogens in the production of polyclonal and monoclonal antibodies, with corresponding changes in reagents and techniques known to those skilled in the art.

Example 15: Purification of Serum Antibodies Which Specifically Bind to CS198 Peptides

20        Immune sera, obtained as described hereinabove in Examples 13 and/or 14, is affinity purified using immobilized synthetic peptides prepared as described in Example 10, or recombinant proteins prepared as described in Example 11. An IgG fraction of the antiserum is obtained by passing the diluted, crude antiserum over a Protein A column (Affi-Gel protein A, Bio-Rad, Hercules, CA). Elution with a  
25      buffer (Binding Buffer, supplied by the manufacturer) removes substantially all proteins that are not immunoglobulins. Elution with 0.1 M buffered glycine (pH 3) gives an immunoglobulin preparation that is substantially free of albumin and other serum proteins.

30        Immunoaffinity chromatography is performed to obtain a preparation with a higher fraction of specific antigen-binding antibody. The peptide used to raise the antiserum is immobilized on a chromatography resin, and the specific antibodies directed against its epitopes are adsorbed to the resin. After washing away non-binding components, the specific antibodies are eluted with 0.1 M glycine buffer, pH 2.3. Antibody fractions are immediately neutralized with 1.0 M Tris buffer (pH 8.0) to preserve immunoreactivity. The chromatography resin chosen depends on the reactive groups present in the peptide. If the peptide has an amino group, a resin

such as Affi-Gel 10 or Affi-Gel 15 is used (Bio-Rad, Hercules, CA). If coupling through a carboxy group on the peptide is desired, Affi-Gel 102 can be used (Bio-Rad, Hercules, CA). If the peptide has a free sulphydryl group, an organomercurial resin such as Affi-Gel 501 can be used (Bio-Rad, Hercules, CA).

5        Alternatively, spleens can be harvested and used in the production of hybridomas to produce monoclonal antibodies following routine methods known in the art as described hereinabove.

Example 16: Western Blotting of Tissue Samples

10        Protein extracts were prepared by homogenizing tissue samples in 0.1 M Tris-HCl (pH 7.5), 15% (w/v) glycerol, 0.2 mM EDTA, 1.0 mM 1,4-dithiothreitol, 10 µg/ml leupeptin, and 1.0 mM phenylmethylsulfonylfluoride (S. R. Kain et al., *Biotechniques* 17:982 (1994)). Following homogenization, the homogenates were centrifuged at 4°C for 5 minutes to separate supernatant from debris. For protein 15 quantitation, 3-10 µl of supernatant were added to 1.5 ml of bicinchoninic acid reagent (Sigma, St. Louis, MO), and the resulting absorbance at 562 nm were measured.

For SDS-PAGE, samples were adjusted to desired protein concentration with Tricine Buffer (Novex, San Diego, CA), mixed with an equal volume of 2X 20 Tricine sample buffer (Novex, San Diego, CA), and heated for 5 minutes at 100°C in a thermal cycler. Samples were then applied to a Novex 10-20% Precast Tricine Gel for electrophoresis. Following electrophoresis samples were transferred from the gels to nitrocellulose membranes in Novex Tris-Glycine Transfer buffer. Membranes were then probed with specific anti-peptide antibodies using the reagents 25 and procedures provided in the Western Lights Plus or Western Lights (Tropix, Bedford, MA) chemiluminescence detection kits. Chemiluminescent bands were visualized by exposing the developed membranes to Hyperfilm ECL (Amersham, Arlington Heights, IL).

Figure 6 shows the results of the Western blot performed on a panel of tissue 30 extracts using antiserum against the CS198 synthetic peptide of SEQUENCE ID NO 44 (see Example 14). Each lane of Figure 6 represents a different tissue protein extract: (1) endometrial cancer; (2) breast cancer; (3) normal lung; (4) benign prostatic hyperplasia; (5) ovarian cancer; (6) normal bladder; (7) bladder cancer; (8- 10) normal colon; (11-13) colon cancer; and (14) markers). A band of 35 approximately 45 kD is seen in all samples; however, the band is more intense in the

-83-

cancerous tissue extracts than in the normal tissue extracts (see lanes 1, 2, 5, 7, 11, 12, and 13).

5 A competition experiment was performed in an analogous manner as above with the following exception: the primary antibody (anti-peptide polyclonal antiserum) was pre-incubated overnight at 4°C with varying concentrations of peptide immunogen prior to exposure to the nitrocellulose filter. Development of the Western was continued as above. Antibody binding to the band of approximately 45 kD was inhibited at a concentration of 2.6  $\mu$ M CS198 synthetic peptide (SEQUENCE ID NO 44).

10 After visualization of the Western on film, bands were also visualized directly on the membrane by the addition and development of chromogenic substrate 5-bromo-4-chloro-3-indolyl phosphate (BCIP). The solution contained 0.016% BCIP, 100 mM NaCl, 5 mM MgCl<sub>2</sub> and 100 mM Tris-HCl, pH 9.5. The filter was incubated in the solution at room temperature until the bands developed to the 15 desired intensity. Molecular mass determination was made based upon the mobility of pre-stained molecular weight standards (Novex, San Diego, CA) and biotinylated molecular weight standards (Tropix, Bedford, MA).

Example 17: EIA Microtiter Plate Assay

20 The immunoreactivity of antiserum preferably obtained from rabbits or mice as described in Example 13 or Example 14 was determined by means of a microtiter plate EIA, as follows. Briefly, synthetic peptides, SEQUENCE ID NO 43, SEQUENCE ID NO 44, SEQUENCE ID NO 45, and SEQUENCE ID NO 46, prepared as described in Example 10, were dissolved in carbonate buffer (50 mM, 25 pH 9.6) to a final concentration of 2  $\mu$ g/ml. Next, 100  $\mu$ l of the peptide or protein solution were placed in each well of an Immulon 2<sup>®</sup> microtiter plate (Dynex Technologies, Chantilly, VA). The plate was incubated overnight at room temperature and then washed four times with deionized water. The wells were blocked by adding 125  $\mu$ l of a suitable protein blocking agent, such as Superblock<sup>®</sup> 30 (Pierce Chemical Company, Rockford, IL), to each well and then immediately discarding the solution. This blocking procedure was performed three times. Antiserum obtained from immunized rabbits or mice, prepared as previously described, was diluted in a protein blocking agent (e.g., a 3% Superblock<sup>®</sup> solution) in PBS containing 0.05% Tween-20<sup>®</sup> (monolaurate polyoxyethylene ether, Sigma 35 Chemical Company, St. Louis, MO) and 0.05% sodium azide at dilutions of 1:100, 1:500, 1:2500, 1:12,500, and 1:62,500 and placed in each well of the coated

micotiter plate. The wells then were incubated for three hours at room temperature. Each well was washed four times with deionized water. One hundred microliters of alkaline phosphatase-conjugated goat anti-rabbit IgG or goat anti-mouse IgG antisera (Southern Biotech, Birmingham, AB) diluted 1:2000 in 3% Superblock<sup>®</sup> solution in phosphate buffered saline containing 0.05% Tween 20<sup>®</sup> and 0.05% sodium azide, were added to each well. The wells were incubated for two hours at room temperature. Next, each well was washed four times with deionized water. One hundred microliters of paranitrophenyl phosphate substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) then were added to each well. The wells were incubated for thirty minutes at room temperature. The absorbance at 405 nm was read in each well. Positive reactions were identified by an increase in absorbance at 405 nm in the test well above that absorbance given by a non-immune serum (negative control). A positive reaction was indicative of the presence of detectable anti-CS198 antibodies. Titers of the anti-peptide antisera were calculated from the previously described dilutions of antisera and defined as the calculated dilution, where A<sub>405nm</sub>=0.5 OD.

Example 18: Coating of Solid Phase Particles

A. Coating of Microparticles with Antibodies Which Specifically Bind to CS198 Antigen. Affinity purified antibodies which specifically bind to CS198 protein (see Example 15) are coated onto microparticles of polystyrene, carboxylated polystyrene, polymethylacrylate or similar particles having a radius in the range of about 0.1 to 20  $\mu\text{m}$ . Microparticles may be either passively or actively coated. One coating method comprises coating EDAC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (Aldrich Chemical Co., Milwaukee, WI) activated carboxylated latex microparticles with antibodies which specifically bind to CS198 protein, as follows. Briefly, a final 0.375% solid suspension of resin washed carboxylated latex microparticles (available from Bangs Laboratories, Carmel, IN or Serodyn, Indianapolis, IN) are mixed in a solution containing 50 mM MES buffer, pH 4.0 and 150 mg/l of affinity purified anti-CS198 antibody (see Example 14) for 15 minutes in an appropriate container. EDAC coupling agent is added to a final concentration of 5.5  $\mu\text{g}/\text{ml}$  to the mixture and mixed for 2.5 hr at room temperature.

The microparticles then are washed with 8 volumes of a Tween 20<sup>®</sup>/sodium phosphate wash buffer (pH 7.2) by tangential flow filtration using a 0.2  $\mu\text{m}$  Microgon Filtration module. Washed microparticles are stored in an appropriate

buffer which usually contains a dilute surfactant and irrelevant protein as a blocking agent, until needed.

B. Coating of 1/4 Inch Beads. Antibodies which specifically bind to CS198-antigen also may be coated on the surface of 1/4 inch polystyrene beads by routine methods known in the art (Snitman et al, US Patent 5,273,882) and used in competitive binding or EIA sandwich assays.

Polystyrene beads first are cleaned by ultrasonication for about 15 seconds in 10 mM NaHCO<sub>3</sub> buffer at pH 8.0. The beads then are washed in deionized water until all fines are removed. Beads then are immersed in an antibody solution in 10 mM carbonate buffer, pH 8 to 9.5. The antibody solution can be as dilute as 1 µg/ml in the case of high affinity monoclonal antibodies or as concentrated as about 500 µg/ml for polyclonal antibodies which have not been affinity purified. Beads are coated for at least 12 hours at room temperature, and then they are washed with deionized water. Beads may be air dried or stored wet (in PBS, pH 7.4). They also may be overcoated with protein stabilizers (such as sucrose) or protein blocking agents used as non-specific binding blockers (such as irrelevant proteins, Carnation skim milk, Superblock®, or the like).

### Example 19: Microparticle Enzyme Immunoassay (MEIA)

20 CS198 antigens are detected in patient test samples by performing a standard antigen competition EIA or antibody sandwich EIA and utilizing a solid phase such as microparticles (MEIA). The assay can be performed on an automated analyzer such as the IMx® Analyzer (Abbott Laboratories, Abbott Park, IL).

25 A. Antibody Sandwich EIA. Briefly, samples suspected of containing  
CS198 antigen are incubated in the presence of anti-CS198 antibody-coated  
microparticles (prepared as described in Example 17) in order to form  
antigen/antibody complexes. The microparticles then are washed and an indicator  
reagent comprising an antibody conjugated to a signal generating compound (i.e.,  
enzymes such as alkaline phosphatase or horseradish peroxide) is added to the  
30 antigen/antibody complexes or the microparticles and incubated. The microparticles  
are washed and the bound antibody/antigen/antibody complexes are detected by  
adding a substrate (e.g., 4-methyl umbelliferyl phosphate (MUP), or OPD/peroxide,  
respectively), that reacts with the signal generating compound to generate a  
measurable signal. An elevated signal in the test sample, compared to the signal  
35 generated by a negative control, detects the presence of CS198 antigen. The

presence of CS198 antigen in the test sample is indicative of a diagnosis of a GI tract disease or condition, such as GI tract cancer.

**B. Competitive Binding Assay.** The competitive binding assay uses a peptide or protein that generates a measurable signal when the labeled peptide is contacted with an anti-peptide antibody coated microparticle. This assay can be performed on the IMx® Analyzer (available from Abbott Laboratories, Abbott Park, IL). The labeled peptide is added to the CS198 antibody-coated microparticles (prepared as described in Example 17) in the presence of a test sample suspected of containing CS198 antigen, and incubated for a time and under conditions sufficient to form labeled CS198 peptide (or labeled protein) / bound antibody complexes and/or patient CS198 antigen / bound antibody complexes. The CS198 antigen in the test sample competes with the labeled CS198 peptide (or CS198 protein) for binding sites on the microparticle. CS198 antigen in the test sample results in a lowered binding of labeled peptide and antibody coated microparticles in the assay since antigen in the test sample and the CS198 peptide or CS198 protein compete for antibody binding sites. A lowered signal (compared to a control) indicates the presence of CS198 antigen in the test sample. The presence of CS198 antigen suggests the diagnosis of a GI tract disease or condition, such as GI tract cancer.

The CS198 polynucleotides and the proteins encoded thereby which are provided and discussed hereinabove are useful as markers of GI tract tissue disease, especially GI tract cancer. Tests based upon the appearance of this marker in a test sample such as blood, plasma or serum can provide low cost, non-invasive, diagnostic information to aid the physician to make a diagnosis of cancer, to help select a therapy protocol, or to monitor the success of a chosen therapy. This marker may appear in readily accessible body fluids such as blood, urine or stool as antigens derived from the diseased tissue which are detectable by immunological methods. This marker may be elevated in a disease state, altered in a disease state, or be a normal protein of the GI tract which appears in an inappropriate body compartment.

-87-

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: ABBOTT LABORATORIES

(ii) TITLE OF THE INVENTION: REAGENTS AND METHODS USEFUL  
FOR DETECTING DISEASES OF THE GASTROINTESTINAL  
TRACT

(iii) NUMBER OF SEQUENCES: 49

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: Abbott Laboratories
- (B) STREET: 100 Abbott Park Road
- (C) CITY: Abbott Park
- (D) STATE: IL
- (E) COUNTRY: USA
- (F) ZIP: 60064-3500

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Diskette
- (B) COMPUTER: IBM Compatible
- (C) OPERATING SYSTEM: DOS
- (D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: 08/828,855
- (B) FILING DATE: 31-MAR-1997

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Becker, Cheryl L.
- (B) REGISTRATION NUMBER: 35,441
- (C) REFERENCE/DOCKET NUMBER: 6065.PC.01

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 847/935-1729
- (B) TELEFAX: 847/938-2623
- (C) TELEX:

-88-

## (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 67 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCTGCCTGCA CCCGCTCACC CTGAGCCCT TGGGGTGGTG GGAGGCCTG GAATCCCCAC	60
TGTGCAG	67

## (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGAGGCTGCA GACTGTGGAG CCGGGAGCCG GCAG	34
---------------------------------------	----

## (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1841 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CAGTAAGCCC AGAGGTCTCC ACCCCACGGG AGGAAGGCTG AGGCCAAGAC CCCGGAAGAG	60
ATGGACCGCG TGACCAAGATA CCCCATCCTG GGCATCCCTC AGGCACACCG TGGCACCGGC	120
CTGGTGTGG ATGGAGACAC CAGCTACACA TACCATCTGG TGTGCATGGG CCCCAGGGC	180
AGCGGCTGGG GCCAGGATGA GCCGAGACA TGGCCCACTG ACCACAGGGC CCAGCAGGGC	240
GTGCAGAGGC AGGGGGTGTG CTACAGCGTG CATGCCTACA CTGGCCAGCC GTCCCCACGG	300
GGGCTCCACT CGGAGAACAG GGAGGATGAG GTTGGCAGG TTTACCGCCT GGGGCCAGG	360
GATGCCACC AGGGACGTCC AACATGGCA CTCCGCCAG AGGACGGGA GGACAAGGAG	420
ATGAAGACCT ACCGCCTGGA TGCTGGGAC GCTGACCCCA GGAGGCTGTG TGACCTGGAG	480
CGGGAGCGCT GGGCCGTAT CCAGGGCCAG GCAGTCAGGA AGAGCAGCAC CGTGGCCACG	540
CTCCAGGGCA CTCTGACCA CGGAGACCCC AGGACCTCCG GCCCCACCTCG GTCCACGCC	600
CTGGAGGAGA ACGTGGTGA CAGGGAGCAG ATTGACTTCC TGGCAGGGAG ACAGCAGTTC	660
CTGAGTCTGG AGCAGGGGAA CAAGGGGGCC CCTCATAGCT CCCCAGGGCAG GGGGACCCCT	720
GCAGGCACAA CCCCAGGGGC CAGCCAGGCC CCCAAGGCCT TCAACAAGCC CCACCTGGCC	780
AACGGGCACG TGTTCCCAT CAAGCCCCAG GTGAAGGGGG TGGTCAGGGAG AGAGAACAAAG	840
GTGCGTGTG TGCCCACCTG GGCCAGTGTG CAAGTTGTGG ATGACCTTGG CTCTTGGCC	900
TCAGTGGAGT CCCCGGGGAC CCCCAAGGAG ACGCCCATCG AGCGGGAGAT CCGTCTGGCT	960
CAGGAGCGTG AGGCAGACCT GCGAGAGCAG AGGGGGCTTC GGCAGGCAAC CGACCACCAAG	1020
GAGCTGGTGG AAATCCCCAC CAGGCCGTG CTGACCAAG TGAGCTGTAT CACAGCCCCA	1080
CGGCGGGAGA GAGGGCGCCC GTCCCTCTAC GTGAGGGGG ACATAGTACA GGAGACACAG	1140
CGTGAGGAAG ACCACCGGGC GGAGGGCTG CACGTGGGCC GGGCGTCCAC ACCCGACTGG	1200
GTCTCGGAGG GTCCCCAGCC CGGACTCCGG AGAGCCCTCA GCTCAGATT CATCCTCAGC	1260
CCGGCCCCAG ATGCCCGTGC GGCCGACCCCA GCTCCAGAAG TGAGGAAGGT GAACCGCAGTC	1320
CCACCTGATG CCTACCAAGCC GTACCTGAGC CCCGGGACCC CCCAGCTAGA ATTCTCAGCC	1380
TTCGGAGCAT TCGGCAAGCC CAGCAGTCTC TCCACAGCGG AGGCCAAGGC TGCGACTTCA	1440
CCAAAGGCCA CGATGTCCCC GAGGCATCTC TCAGAATCCT CTGGAAAACC CCTGAGCACA	1500
AAGCAAGAGG CATCGAAGCC CCCTCGGGGA TGCCCGCAAG CCAACAGGGG TGTCGTGCGG	1560
TGGGAGTACT TCCGCTGCG TCCTCTGCGG TTCAGGGCCC CAGACGAGCC CCAGCAGGCC	1620
CAAGTCCCCC ATGTCTGGGG CTGGGAGGTG GCTGGGGCCC CTGCACTGAG GCTGGCAGAAC	1680

-89-

TCCCCAGTCAT	CTGATCTGCT	GGAAAGGGAG	AGGGAGAGTG	TCCTGCGCCG	GGAGCAAGAG	1740
GTGGCAGAGG	AGCAGAGAAA	TGCTCTCTTC	CCAGAGGTCT	TCTCCCCAAC	GCCAGATGAG	1800
AACTCTGACC	AGAACTCCAG	GAGCTCCTCC	CAGGCATCCG	G		1841

## (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 132 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGCATCACGG	GCAGTTACTC	GGTGTCTGAG	TCTCCCTTCT	TCAGCCCCAT	CCACCTACAC	60
TCAAACGTGG	CGTGGACAGT	GGAAAGATCCA	GTGGACAGTG	CTCCTCCCCG	GCAGAGAAAG	120
AAGGAGCAAT	GG					132

## (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTACGCTGGC	ATCAAACCCCT	CGGACGGTAT	CAACTCAGAG	GT		42
------------	-------------	------------	------------	----	--	----

## (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 820 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGGTCCCTGGA	AGGCATACGG	GTGACCGTC	ACAAGAACGC	CATGGCAGAG	CGCTGGGAAT	60
CCCGCATCTA	CGCCAGTGAG	GAGGATGACT	GAGCCTCGGG	ATGGGGCGCC	CACCCCTGCA	120
CCTGCCCTGGA	CCCTCGTGGG	AACTGCCAAG	ACCATCGCCA	AGCCCCCACC	CTAGGAAATG	180
GGTCCTAGGT	CCAGGATCCA	AGAACCCACAG	CTCATCTGCC	AAACAATCCA	CCATGGGCAC	240
ATTTGGGACT	GTTGGGTTTT	TCGTTTCCGT	TTCTATCTTC	CTTTAGAAAT	GTTTCTGCCT	300
TTGGGGTCTA	AAGCTTTGG	GGATGAAATG	GGACCCCTGC	TGATTCTTTC	TGCTTCTAAG	360
ACTTTGCCAA	ATGCCCTGGG	TCTAAGAAAG	AAAGAGACCC	GCTCCTCCAC	TTTCAGGTGT	420
AATTGCTTC	CGCTAGTCTG	AGGGCAGAGG	GACCCTGCAA	AGAGGGTGGC	ACAGATCGCA	480
GCACCTTGAG	GGGCGCGGG	TCTGAGGGAG	GAGACACTCA	GCTCCTCCCT	CTGAGAAGTC	540
CCAAGCTGAG	AGGGGAGACC	TGCCCCCTTC	CAACCCCTGGG	AAACCATCCA	GTCTGAGGGA	600
GGAGGCCAAA	CTCCCAGTGC	TGGGGGTCCC	TGTGCAGGCC	TCAAACCCCT	CACCTTGGTG	660
CACCCAGCCA	CACCTGGTGG	ACACAAAGCT	CTCACATCGA	TAGGATCCA	TGAGGATGGT	720
CCCCTTCACC	TGGGAGAAAA	GTGACCCAGT	TTAGGAGCTG	GAGGGGGGTC	TTTGTCCCCC	780
ACCCCCAAAC	TGCCCTGAAA	TAACACCTGGA	GTGAGCTGCC			820

## (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 281 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-90-

- (ix) FEATURE:
  - (A) NAME/KEY: base\_polymorphism
  - (B) LOCATION: 25
  - (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"
- (ix) FEATURE:
  - (A) NAME/KEY: base\_polymorphism
  - (B) LOCATION: 86
  - (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"
- (ix) FEATURE:
  - (A) NAME/KEY: base\_polymorphism
  - (B) LOCATION: 87
  - (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"
- (ix) FEATURE:
  - (A) NAME/KEY: base\_polymorphism
  - (B) LOCATION: 131
  - (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"
- (ix) FEATURE:
  - (A) NAME/KEY: base\_polymorphism
  - (B) LOCATION: 155
  - (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"
- (ix) FEATURE:
  - (A) NAME/KEY: base\_polymorphism
  - (B) LOCATION: 254
  - (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"
- (ix) FEATURE:
  - (A) NAME/KEY: base\_polymorphism
  - (B) LOCATION: 259
  - (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCTGCCTGCA	CCCGCTCACC	CTGANCGCCT	TGGGGTGGTG	GGAGGGCGCTG	GAATCCCCAC	60
TGTGAGTAA	GCCCCAGATGT	CTCCANCCA	ACGGTAGGAA	GGCTGATGCC	AAGACCCCGG	120
AAGAGATGGA	NCGCGTGACC	AGATACCCAT	CCTGNGCATC	CCTCAGGCAC	ACCGTGGCAC	180
CGGCCTGGTG	CTGGATGGAG	ACACCAGCTA	CACATACCAT	CTGGTGTGCA	TTGGCCCCGA	240
AGCCAGCGGC	TGGNGCCANG	ATGAGCCGCA	ACATGGCCAC	T		281

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 294 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ix) FEATURE:
  - (A) NAME/KEY: base\_polymorphism
  - (B) LOCATION: 45
  - (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"
- (ix) FEATURE:
  - (A) NAME/KEY: base\_polymorphism

-91-

(B) LOCATION: 195  
 (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

(ix) FEATURE:  
 (A) NAME/KEY: base\_polymorphism  
 (B) LOCATION: 207  
 (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

(ix) FEATURE:  
 (A) NAME/KEY: base\_polymorphism  
 (B) LOCATION: 212  
 (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

(ix) FEATURE:  
 (A) NAME/KEY: base\_polymorphism  
 (B) LOCATION: 243  
 (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

(ix) FEATURE:  
 (A) NAME/KEY: base\_polymorphism  
 (B) LOCATION: 286  
 (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCTGCCTGCA CCCGCTTCAC CCTGAGCGCC TTGGGGTGGT GGGANGCGCT GGAATCCCCA	60
CTGTGCAGTA AGCCCAGAGG TCTCCACCCC ACGGGATGAA GGCTGAGGCC AAGACCCCCGG	120
AAGAGATGGA CCGCGTGACC AGATACCCCA TCCTGGGCAT CCCTCAGGCC CACCGTGGGC	180
ACCGGCTGG TGCTNGATGG AGACACNAGT TNCACATACC ATCTGGTGTG CATGGGCC	240
GANGCAGCGG TGGGGCAGGA TGAGCCGCAG ACATGGCCAC TGACCNAAGG CCAT	294

## (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 151 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGAGGGCTGCA GACTGTGGAG CCGGGAGCCG GCAGTAAGCC CAGAGGTCTC CACCCACGG	60
GAGGAAGGCT GAGGCCAAGA CCCCGGAAGA GATGGACCGC GTGACCAGAT ACCCCATCCT	120
GGGCATCCCT CAGGCCACACC GTGGCACCGG T	151

## (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 118 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGCAGACTGT GGAGCCGGGA GCCGGCAGTA AGCCCAGAGG TCTCCACCCC ACGGGAGGAA	60
GGCTGAGGCC AAGACCCCCGG AAGAGATGGA CCGCGTGACC AGATACCCCA TCCTGGGC	118

- 92 -

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 223 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TGCAGACTGT GGAGCCGGGA GCGGGCAGTA AGCCCAGAGG TCTCCACCCC ACGGGAGGAA	60
GGCTGAGGCC AAGACCCCGG AAGAGATGGA CCGCGTGACC AGATACCCCA TCCTGGGCAT	120
CCCTCAGGCA CACCGTGGCA CCGGCCTGGT GCTGGATGGA GACACCAGCT ACACATACCA	180
TCTGGTGTGC ATGGGCCCCG AGGCCAGCGG TGGGGCCAGG ATG	223

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 257 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCCCGGAAGA GATGGACCGC GTGACCAGAT ACCCCATCCT GGGCATCCCT CAGGCACACC	60
GTGGCACCCG CCTGGTGCTG GATGGAGACA CCAGCTACAC ATACCATCTG GTGTGCATGG	120
GCCCCGAGGC CAGCGGCTGG GGCCAGGATG AGCCGCAGAC ATGGCCCACT GACCACAGGG	180
CCCAGCAGGG CGTGCAGAGG CAGGGGTGT CCTACAGCGT GCATGCCTAC ACTGGCCAGC	240
CGTCCCCACG GGGGCTC	257

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TAGGGCCAGC GGCTGGGGCC AGGATGAGCC GCAGACATGG CCCACTGACC ACAGGGCCCA	60
GCAGGGCGTG CAGAGGCAGG GGGTGTCTA CAGCGTGCAT GCCTACACTG GCCAGCCGTC	120
CCCACGGGGG CTCCACTCGG AGAACAGGGA GGATGAGGGT TGGCAGGTTT ACCGCCTGGG	180
CGCCAGGGAT GCCCACCAGG GACGTCAAC ATGGCACTC CGCCCAGAGG ACGGGGAGGA	240
CAAGGAGATG AAGACCTACC GCCTGGATGC TGGGGACGCT GACCCCAGGA GGCTGTGTGA	300
CTGGAG	306

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 261 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCTGTCTCGC TGCCAGGAAG TCAATCTGCT CCCTGTCAAC CACGTTCTCC TCCAGGGGCG	60
TGGACCGAGG TGGGCCGGGG GTCCCTGGGT CTCCGTGGTC AGGAGTGCCT TGGAGCGTGG	120
CCACGGTGCT GCTCTTCCTG ACTGCCTGGC CCTGGATGAC GGCCCAGCGC TCCCGCTCCA	180
GGTCACACAG CCTCCTGGGG TCAGCGTCCC CAGCATCCAG GCGGTAGGTC TTCATCTCCT	240

-93-

TGTCCCTCCCC GTCCTCTGGGG C

261

## (2) INFORMATION FOR SEQ ID NO:15:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 295 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (A) NAME/KEY: base\_polymorphism
- (B) LOCATION: 41
- (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGAGCAGGGCG AACAAAGGGGG CCCCTCATAG CTCCTCCGGCC NGGGGGACCC CTGCAGGCAC	60
AACCCCAGGG GCCAGCCAGG CCCCCAAGGC CTTCAACAAG CCCCACCTGG CCAACGGGCA	120
CGTGGTTCCC ATCAAGCCCC AGGTGAAGGG GGTGGTCAGG GAAGAGAACAA AGGTGCGTGC	180
TGTGCCCAACC TGGGCCAGTG TCCAAGTTGT GGATGACCCCT GGCTCCTTGG CCTCAGTGGAA	240
GTCCCCGGGG ACCCCAAGG AGACGCCAT CGAGCGGGAG ATCCGTCTGG CTCAG	295

## (2) INFORMATION FOR SEQ ID NO:16:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 292 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (A) NAME/KEY: base\_polymorphism
- (B) LOCATION: 9
- (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

## (ix) FEATURE:

- (A) NAME/KEY: base\_polymorphism
- (B) LOCATION: 260
- (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TGCAGCGGNG ACATAGTACA GGAGACACAG CGTGAGGAAG ACCACCGGCG GGAGGGCCTG	60
CACGTGGGCC GGGCGTCCAC ACCCGACTGG GTCTCGGAGG GTCCCCAGCC CGGACTCCGG	120
AGAGCCTCA GCTCAGATTG CATCCTCAGC CCGGCCCCAG ATGCCCGTGC GGCCGACCCA	180
GCTCCAGAAG TGAGGAAGGT GAACCGCAGTC CCACCTGATG CCTACCAGCC GTACCTGAGC	240
CCCGGGACCC CCCAGCTAGT ATTCTCAGCT TCGGAGCATT CGGCAAGCCC AG	292

## (2) INFORMATION FOR SEQ ID NO:17:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 226 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (A) NAME/KEY: base\_polymorphism
- (B) LOCATION: 89
- (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

- 94 -

(ix) FEATURE:  
 (A) NAME/KEY: base\_polymorphism  
 (B) LOCATION: 101  
 (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTGATGCCCTA	CCAGCCGTAC	CTGAGCCCCG	GGACCCCCCA	GCTAGAATTC	TCAGCCTTCG	60
GAGCATTCTGG	CAAGCCCAGC	AGTCTCTCNA	CAGCGGAGGA	NCAAGGCTGC	GACTTCACCA	120
AAGGCCACGA	TGTCCCCGAG	GCATCTCTCA	GAATCCTCTG	GAAAACCCCT	GAGCACAAAG	180
CAAGAGGCAT	CGAAGCCCCC	TCGGGGATGC	CCGCAAGCCA	ACAGGG		226

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 206 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTCTCACACAG	CGGAGGCCAA	GGCTGCGACT	TCACCAAAGG	CCACGATGTC	CCCGAGGCAT	60
CTCTCAGAAT	CCTCTGGAAA	ACCCCTGAGC	ACAAAGCAAG	AGGCATCGAA	GCCCCCTCGG	120
GGATGCCCGC	AAGCCAACAG	GGGTGTCGTG	CGGTGGGAGT	ACTTCCGCCT	GCGTCCTCTG	180
CGGTTCAAGGG	CCCCAGACGA	GCCCCA				206

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 258 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ix) FEATURE:

(A) NAME/KEY: base\_polymorphism  
 (B) LOCATION: 84  
 (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CGGGGATGCC	CGCAAGCCAA	CAGGGGTGTC	GTGCGGTGGG	AGTACTTCCG	CCTGCGTCCT	60
CTGCGGTTCA	GGGCCCCAGA	CGANCCCAG	CAGGCCAAG	TCCCCCATGT	CTGGGGCTGG	120
GAGGTGGCTG	GGGCCCCCTGC	ACTGAGGCTG	CAGAAGTCCC	AGTCATCTGA	TCTGCTGGAA	180
AGGGAGAGGG	AGAGTGTCT	GCGCCGGAG	CAAGAGGTGG	CAGAGGAGCG	GAGAAATGCT	240
CTCTTCCCAG	AGGTCTTC					258

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 233 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ix) FEATURE:

(A) NAME/KEY: base\_polymorphism  
 (B) LOCATION: 19  
 (D) OTHER INFORMATION: /note= "'N' represents an A or G or T or C polymorphism at this position"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

-95-

GGAGGTGGCT	GGGGCCCCNG	CACTGAGGCT	GCAGAAGTCC	CAGTCATCTG	ATCTGCTGGA	60
AAGGGAGAGG	GAGAGTGTCC	TGCGCCGGGA	GCAAGAGGTG	GCAGAGGAGC	GGAGAAATGC	120
TCTCTTCCA	GAGGTCTTCT	CCCCAACGCC	AGATGAGAAC	TCTGACCAGA	ACTCCAGGAG	180
CTCCTCCAG	GCATCCGGCA	TCACGGCAG	TTATCGGTGT	CTGAGTCTCC	CTT	233

## (2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CTCCCTTCTT	CAGCCCCATC	CACCTACACT	CAAACGTGGC	GTGGACAGTG	GAAGATCCAG	60
TGGACAGTGC	TCCTCCCGGG	CAGAGAAAGA	AGGAGCAATG	GTACGCTGGC	ATCAACCCCT	120
CGGACGGTAT	CAACTCAGAG	GTCCTGGAAG	CCATACGGGT	GACCCGTAC	AAGAACGCCA	180
TGGCAGAGCG	CTGGGAATCC	CGCATCTACG	CCAGTGAGGA	GGATGACTGA	GCCTCGGGAT	240
GGGGCGCCCA	CCCCCTGCC	TGCCCTGACC	CTCGTGG			277

## (2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 284 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: base\_polymorphism
- (B) LOCATION: 56
- (D) OTHER INFORMATION: /note= "N' represents an A or G or T or C polymorphism at this position"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CTGGGAATCC	CCCATCTACG	CCAGTGAGGA	GGATGACTGA	GCCTCGGGAT	GGGCNCCCA	60
CCCCCTGCC	TGCCCTGACC	CTCGTGGAA	CTGCAAGAC	CATGCCAAG	CCCCCACCC	120
AGGAAATGGG	TCCTAGGTCC	AGGATCCAAG	AACCACAGCT	CATCTGCCAA	CAATCCCACC	180
ATGGGCACAT	TTGGGACTGT	TGGGTTTTTC	GTTCCTGTT	CTATCTCCT	TTAGAAATGT	240
TTCTGCCTT	GGGTCTAAA	GCTTTGGGG	ATGAAATGGG	ACCC		284

## (2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 324 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GCTTTTGGGG	ATGAAATGGG	ACCCCTGCTG	ATTCTTTCTG	CTTCTAAGAC	TTTGCCAAT	60
CCCCCTGGTC	TAAGAAAGAA	AGAGACCGC	TCCTCCACTT	TCAGGTGTAA	TTTGTCTCCG	120
CTAGTCTGAG	GGCAGAGGG	CCGGTCAAAG	AGGGTGGCAC	AGATCGCAGC	ACCTTGAGGG	180
GCTGCAGGTC	TGAGGGAGGA	GACACTCAGC	TCCTCCCTCT	GAGAAGTCCC	AAGCTGAGAG	240
GGGAGACCTG	CCCCTTCCA	ACCCCTGGAA	ACCATCCAGT	CTGAGGGAGG	AGGCCAAACT	300
TCCAGTGCTG	GGGGTCCCTG	TGCA				324

## (2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

-96-

- (A) LENGTH: 266 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GAAAGAAAGA GACCCGCTCC	TCCACTTCA GGTGTAATT	GCTTCCGCTA GTCTGAGGGC	60
AGAGGGACCG	GTCAAAGAGG	GTGGCACAGA TCGCAGCACC	120
GGGAGGAGAC	ACTCAGCTCC	TTGAGGGGCT GCGGGTCTGA	180
CTTTCCAACC	CTGGGAAACC	AAGTCCAAG CTGAGAGGGG	240
GTCCCTGTGC	ATCCAGTCTG	AGACCTGCC	266
		CAAACCTCCC AGTGCTGGGG	
		GTCCCTGTGC AGCCCTCAAA CCCTTC	

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 196 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGTCCCTGTG CAGCCCTCAA	ACCCCTCACC TTGGTGCACC	CAGCCACACC TGGTGGACAC	60
AAAGCTCTCA	CATCGATAGG ATCCCATGAG	GATGGTCCCC TTCACCTGGG	120
CCCAGTTTAG	GAGCTGGAGG GGGGTCTTTG	AGAAAAGTGA CCAAACGTGCC	180
CCTGGAGTGA	GCTGCC	CTGAAATAAA	196

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1547 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GTCGACCCAC GCGTCCGCC	ACCGTCCGC	GGACCGTGG GCTGATGCCT	ACCAGCCGTA	60
CCTGAGCCCC	GGGACCCCC	AGCTAGAATT CTCAGCCTTC	GGAGCATTG GCAAGCCAG	120
CAGTCCTCTCC	ACAGCGGAGG	CCAAGGCTGC	GACTTCACCA AAGGCCACGA	180
GCATCTCTCA	GAATCCTCTG	GAAAACCCCT	CGACACAAAG CAAGAGGCAT	240
TCGGGGATGC	CCGCAAGCCA	ACAGGGGTGT	TGTCCCGAG CGAAGCCCCC	300
TCTCGGGTTC	AGGGCCCCAG	CGAGGCCCCA	GAGTACTTCC GCCTGCGTCC	360
GGAGGTGGCT	GGGGCCCCCTG	CACTGAGGCT	GTCCCCCATG TCTGGGGCTG	420
AAGGGAGAGG	GAGAGTGTCC	TGCGCCGGGA	CACTGAGGCT GCAGAGGAGC	480
TCTCTCCCA	GAGGTCTTCT	CCCCAACGCC	GGAGAAATGC AGATGAGAAC	540
CTCCTCCAG	GCATCCGGCA	TCACGGGAG	TCTGACCAAG ACTCCAGGAG	600
CCCCATCCAC	CTACACTCAA	ACGTGGCGTG	TCTGAGTCTC CCTTCTTCAG	660
TCCCGGGCAG	AGAAAGAAGG	AGCAATGGTA	GACAGTGGAA GATCCAGTGG	720
CTCAGAGGTC	CTGGAAGCCA	CGCTGGCATC	ACAGTGTCAA CGCTGGCATC	780
GGAAATCCCGC	ATCTACGCCA	AAACGGGTGAC	AACGCCATGG CAGAGCGCTG	840
CCTGCCCTGC	CCTGACCCCTC	GTGGGAACCTG	TCGGGATGGG GCGCCCACCC	900
AAATGGGTCC	TAGGTCCAGG	CCAAGACCAT	CGCCAAGCCC CCACCCCTAGG	960
GGCACATTG	GGACTGTTGG	CGCTGGCAT	ACCGTATCAA CGCTGGCAT	1020
TGCCTTGGG	GTCTAAAGCT	TTTGGGATG	AAATGGGACC CCTGCTGATT	1080
CTAAGACTTT	GCCAAATGCC	CTGGGTCTAA	CTTGCTGATT CTTTCTGCTT	1140
GGTGTAAATT	GCTTCCGCTA	GTCTGAGGGC	GAAAGAAGA GACCCGCTCC	1200
TCGCAGCACC	TTGAGGGGCT	AGAGGGACCG	TCCACTTTCA GTCAAAGAGG	1260
AAGTCCAAG	CTGAGAGGGG	GTGAGTCTGC	AGCTCAGTCTC GTGGCACAGA	1320
AGGGAGGAGG	CCAAACCTCCC	CTTCCAACC	CTGGGAAACCC ATCCAGTCTG	1380
TGGTGCACCC	AGCACACCT	AGTGTGGACACA	AGCCTCTCAA CCCTTCACCT	1440
ATGGTCCCCCT	TCACCTGGGA	GGTGGACACA	AGCTCTCAC ATCGATAGGA	1500
		AAAGAGTGCAC	TCCCACCATG AGCTGGAGGG	
		CCAGTTAGG	GGGTCTTGT	

- 97 -

CCCCCACCCC CAAACTGCC TGAAATAAAC CTGGAGTGAG CTGCCA

1547

## (2) INFORMATION FOR SEQ ID NO:27:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2894 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTTGCCTGCA	CCCGCTCACC	CTGAGCGCCT	TGGGGTGGTT	GCAGACTGTG	GAGCCGGGAG	60
CGGCAGTAA	GCCCAGAGGT	CTCCACCCCA	CGGGAGGAAG	GCTGAGGCCA	AGACCCCGGA	120
AGAGATGGAC	CGCGTGACCA	GATACCCCAT	CTTGGGCATC	CCTCAGGCAC	ACCGTGGCAC	180
CGGCCTGGTG	CTGGATGGAG	ACACCACTA	CACATACCAT	CTGGTGTGCA	TGGGCCCCGA	240
GGCCAGCGGC	TGGGCCAGG	ATGAGCCGC	GACATGCC	ACTGACCACA	GGGCCCAGCA	300
GGGCGTCAG	AGGCAGGGGG	TGTCCTACAG	CGTGCATGCC	TACACTGGCC	AGCCGTCCCC	360
ACGGGGGCTC	CACTCGGAGA	ACAGGGAGGA	TGAGGGTTGG	CAGGTTTACC	GCCTGGCGC	420
CAGGGATGCC	CACCAAGGGAC	GTCCAACATG	GGCACTCCGC	CCAGAGGACG	GGGAGGACAA	480
GGAGATGAAG	ACCTACCGCC	TGGATGCTGG	GGACGCTGAC	CCCAGGAGGC	TGTGTGACCT	540
GGAGCGGGAG	CGCTGGCCG	TCATCCAGGG	CCAGGCAGTC	AGGAAGAGGA	GCACCGTGGC	600
CACGCTCCAG	GGCACTCCCTG	ACCACGGAGA	CCCCCAGGACC	CCCAGGCCAC	CTCGGTCCAC	660
GCCCCCTGGAG	GAGAACGCTGG	TTGACAGGGGA	GCAGATTGAC	TTCCTGGCAG	CGAGACAGCA	720
GTTCTTGAGT	CTGGAGCAGG	CGAACAACTGG	GGCCCCCTCAT	AGCTCCCCGG	CCAGGGGGAC	780
CCCTGCAGGC	ACAACCCCAAG	GGGGCAGCGA	GGCCCCCAAG	CCCTTCAACA	AGCCCCCACCT	840
GGCCAACGGG	CACGTGGTTC	CCATCAAGCC	CCAGGTGAAG	GGGGTGTCA	GGGAAGAGAA	900
CAAGGTGCGT	GCTGTGCCCA	CCTGGGCCAG	TGTCCAAGTT	GTGGATGACC	CTGGCTCCCT	960
GGCCTCAGTG	GAGTCCCCGG	GGACCCCCAA	GGAGACGCC	ATCGAGCAGG	AGATCCGTCT	1020
GGCTCAGGAG	CGTGAGGCAG	ACCTGCGAGA	GCAGAGGGGG	CTTCGGCAGG	CAACCGACCA	1080
CCAGGAGCTG	GTGGAATCC	CCACCAGGCC	GCTGCTGACC	AAGCTGAGCC	TGATCACAGC	1140
CCCACGGCGG	GAGAGAGGGC	GCCCCTCCCT	CTACGTGCAG	CGGGACATAG	TACAGGAGAC	1200
ACAGCGTGAG	GAAGACCACC	GGCGGGAGGG	CTTGACGTG	GGCCGGGGCGT	CCACACCCGA	1260
CTGGGTCTCG	GAGGGTCCCC	AGCCCCGGACT	CCGGAGAGCC	CTCAGCTCAG	ATTCCATCCT	1320
CAGGGGGGCC	CCAGATGCC	GTGGGGCGA	CCCAGCTCCA	GAAGTGGAGGA	AGGTGAACCG	1380
CATCCCACCT	GATGCTTACCC	AGCGCTACCT	GAGCCCCGGG	ACCCCCCAGC	TAGAATTCTC	1440
AGCCTTCGGA	GCATTCGGCA	AGCCCAGCAG	TCTCTCCACA	GGGGAGGCCA	AGGCTGCGAC	1500
TTCACCAAAAG	GCCACGATGT	CCCCGAGGCC	TCTCTCAGAA	TCCTCTGGAA	AAACCCCTGAG	1560
CACAAAGCAA	GAGGCATCGA	AGCCCCCTCG	GGGATGCCCG	CAAGCCAACA	GGGGTGTGCGT	1620
GCGGTGGGAG	TACTTCCGCC	TGCGTCCCT	GGCGGTCAGG	GCCCCAGACG	AGCCCCAGCA	1680
GGCCAAGTC	CCCCATGTCT	GGGGCTGGGA	GGTGGCTGGG	GGCCCTGCAC	TGAGGCTGCA	1740
GAAGTCCCGAG	TCATCTGATC	TGCTGGAAAG	GGAGAGGGAG	AGTGTCTG	GGCGGGGAGCA	1800
AGAGGTGGCA	GAGGAGCGGA	GAAATGCTCT	CTTCCCAGAG	GTCTTCTCCC	CAACGCCAGA	1860
TGAGAACACT	GACCGAGAAC	CCAGGAGCTC	CTTCCCAGGCC	TCCGGCATCA	GGGGCAGTAA	1920
CTCGGTGCT	GAGTCTCCCT	TCTTCAGCCC	CATCCACCTA	CACTCAAACG	TGGCGTGGAC	1980
AGTGGAAAGAT	CCAGTGGACA	GTGCTCTCC	CGGGCAGAGA	AAGAAGGAGC	AATGGTACGC	2040
TGGCATCAAC	CCCTCGGACG	GTATCAACTC	AGAGGTCTG	GAAGCCATAC	GGGTGACCCG	2100
TCACAAGAAC	GCCATGGCAG	AGCGCTGGGA	ATCCCCATC	TACGCCAGTG	AGGAGGATGA	2160
CTGAGCCTCG	GGATGGGGCG	CCCACCCCCCT	GCCCTGCCCT	GACCCTCGTG	GGAAACTGCCA	2220
AGACCATCGC	CAAGCCCCCA	CCCTAGGGAA	TGGGTCTAG	GTCCAGGATC	CAAGAACCCAC	2280
AGCTCATCTG	CCACAACATCC	CACCATGGC	ACATTGGGA	CTGTTGGGTT	TTTCGTTTCC	2340
GTTTCTATCT	TCCTTTAGAA	ATGTTCTGC	CTTGGGGTC	TAAAGCTTT	GGGGATGAAA	2400
TGGGACCCCT	GCTGATTCTT	TCTGCTCTA	AGACCTTGCG	AAATGCCCTG	GGTCTAAGAA	2460
AGAAAGAGAC	CCGCTCTCC	ATCTTCAGGT	GTAATTGCT	TCCGCTAGTC	TGAGGGCAGA	2520
GGGACCGGTC	AAAGAGGGTG	GCACAGATCG	CAGCACCTTG	AGGGGCTGCG	GGTCTGAGGG	2580
AGGAGACACT	CAGCTCTCC	CTCTGAGAG	TCCCAAGCTG	AGAGGGGAGA	CCTGCCCTT	2640
TCCAACCTG	GGAAACCATC	CAGTCTGAGG	GAGGAGGCCA	AACTCCCAGT	GCTGGGGTC	2700
CCTGTGCAGC	CCTCAAACCC	TTCACCTTGG	TGCACCCAGC	CACACCTGGT	GGACACAAAG	2760
CTCTCACATC	GATAGGATCC	CATGAGGATG	GTCCCCCTCA	CCTGGGAGAA	AAGTGACCCCA	2820
GTTTAGGAGC	TGGAGGGGGG	TCTTTGTC	CCACCCCCAA	ACTGCCCTGA	AATAAACCTG	2880
GAGTGAGCTG	CCCA					2894

## (2) INFORMATION FOR SEQ ID NO:28:

## (i) SEQUENCE CHARACTERISTICS:

-98-

- (A) LENGTH: 68 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AGCTCGGAAT TCCGAGCTTG GATCCTCTAG AGCGGCCGCC GACTAGTGAG CTCGTCGACC	60
CGGGAATT	68

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 68 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AATTAATTCC CGGGTCGACG AGCTCACTAG TCGGCAGGCCG CTCTAGAGGA TCCAAGCTCG	60
GAATTCCG	68

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AGCGGATAAC AATTCACAC AGGA	24
---------------------------	----

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TGTAAAACGA CGGCCAGT	18
---------------------	----

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CAGAAGTCCC AGTCATCTGA TC	22
--------------------------	----

(2) INFORMATION FOR SEQ ID NO:33:

- 99 -

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

AGAGGTCTCG GAAGCCATAC

20

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGGACTGTTG GGTTTTTCGT

20

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GTCAAAGAGG GTGGCACAGA

20

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CTTTGTGTCC ACCAGGTGTG

20

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

TCTGTGCCAC CCTCTTTGAC

20

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- 100 -

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

ATCCTCCTCA CTGGCGTAGA

20

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

AGAGTTCTCA TCTGGCGTTG

20

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GAATCCTCTG GAAAACCCCT GAGC

24

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CCATTGCTCC TTCTTTCTCT GCCC

24

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 679 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Met	Asp	Arg	Val	Thr	Arg	Tyr	Pro	Ile	Leu	Gly	Ile	Pro	Gln	Ala	His
1				5					10				15		
Arg	Gly	Thr	Gly	Leu	Val	Leu	Asp	Gly	Asp	Thr	Ser	Tyr	Thr	Tyr	His
				20				25					30		

- Leu Val Cys Met Gly Pro Glu Ala Ser Gly Trp Gly Gln Asp Glu Pro  
     35                 40                 45  
 Gln Thr Trp Pro Thr Asp His Arg Ala Gln Gln Gly Val Gln Arg Gln  
     50                 55                 60  
 Gly Val Ser Tyr Ser Val His Ala Tyr Thr Gly Gln Pro Ser Pro Arg  
     65                 70                 75                 80  
 Gly Leu His Ser Glu Asn Arg Glu Asp Glu Gly Trp Gln Val Tyr Arg  
     85                 90                 95  
 Leu Gly Ala Arg Asp Ala His Gln Gly Arg Pro Thr Trp Ala Leu Arg  
     100                105                110  
 Pro Glu Asp Gly Glu Asp Lys Glu Met Lys Thr Tyr Arg Leu Asp Ala  
     115                120                125  
 Gly Asp Ala Asp Pro Arg Arg Leu Cys Asp Leu Glu Arg Glu Arg Trp  
     130                135                140  
 Ala Val Ile Gln Gly Gln Ala Val Arg Lys Ser Ser Thr Val Ala Thr  
     145                150                155                160  
 Leu Gln Gly Thr Pro Asp His Gly Asp Pro Arg Thr Pro Gly Pro Pro  
     165                170                175  
 Arg Ser Thr Pro Leu Glu Glu Asn Val Val Asp Arg Glu Gln Ile Asp  
     180                185                190  
 Phe Leu Ala Ala Arg Gln Gln Phe Leu Ser Leu Glu Gln Ala Asn Lys  
     195                200                205  
 Gly Ala Pro His Ser Ser Pro Ala Arg Gly Thr Pro Ala Gly Thr Thr  
     210                215                220  
 Pro Gly Ala Ser Gln Ala Pro Lys Ala Phe Asn Lys Pro His Leu Ala  
     225                230                235                240  
 Asn Gly His Val Val Pro Ile Lys Pro Gln Val Lys Gly Val Val Arg  
     245                250                255  
 Glu Glu Asn Lys Val Arg Ala Val Pro Thr Trp Ala Ser Val Gln Val  
     260                265                270  
 Val Asp Asp Pro Gly Ser Leu Ala Ser Val Glu Ser Pro Gly Thr Pro  
     275                280                285  
 Lys Glu Thr Pro Ile Glu Arg Glu Ile Arg Leu Ala Gln Glu Arg Glu  
     290                295                300  
 Ala Asp Leu Arg Glu Gln Arg Gly Leu Arg Gln Ala Thr Asp His Gln  
     305                310                315                320  
 Glu Leu Val Glu Ile Pro Thr Arg Pro Leu Leu Thr Lys Leu Ser Leu  
     325                330                335  
 Ile Thr Ala Pro Arg Arg Glu Arg Gly Arg Pro Ser Leu Tyr Val Gln  
     340                345                350  
 Arg Asp Ile Val Gln Glu Thr Gln Arg Glu Glu Asp His Arg Arg Glu  
     355                360                365  
 Gly Leu His Val Gly Arg Ala Ser Thr Pro Asp Trp Val Ser Glu Gly  
     370                375                380  
 Pro Gln Pro Gly Leu Arg Arg Ala Leu Ser Ser Asp Ser Ile Leu Ser  
     385                390                395                400  
 Pro Ala Pro Asp Ala Arg Ala Ala Asp Pro Ala Pro Glu Val Arg Lys  
     405                410                415  
 Val Asn Arg Ile Pro Pro Asp Ala Tyr Gln Pro Tyr Leu Ser Pro Gly  
     420                425                430  
 Thr Pro Gln Leu Glu Phe Ser Ala Phe Gly Ala Phe Gly Lys Pro Ser  
     435                440                445  
 Ser Leu Ser Thr Ala Glu Ala Lys Ala Ala Thr Ser Pro Lys Ala Thr  
     450                455                460  
 Met Ser Pro Arg His Leu Ser Glu Ser Ser Gly Lys Pro Leu Ser Thr  
     465                470                475                480  
 Lys Gln Glu Ala Ser Lys Pro Pro Arg Gly Cys Pro Gln Ala Asn Arg  
     485                490                495  
 Gly Val Val Arg Trp Glu Tyr Phe Arg Leu Arg Pro Leu Arg Phe Arg  
     500                505                510  
 Ala Pro Asp Glu Pro Gln Gln Ala Gln Val Pro His Val Trp Gly Trp  
     515                520                525  
 Glu Val Ala Gly Ala Pro Ala Leu Arg Leu Gln Lys Ser Gln Ser Ser  
     530                535                540  
 Asp Leu Leu Glu Arg Glu Arg Glu Ser Val Leu Arg Arg Glu Gln Glu  
     545                550                560

-102-

- Val Ala Glu Glu Arg Arg Asn Ala Leu Phe Pro Glu Val Phe Ser Pro  
       565                   570                   575  
 Thr Pro Asp Glu Asn Ser Asp Gln Asn Ser Arg Ser Ser Gln Ala  
       580                   585                   590  
 Ser Gly Ile Thr Gly Ser Tyr Ser Val Ser Glu Ser Pro Phe Phe Ser  
       595                   600                   605  
 Pro Ile His Leu His Ser Asn Val Ala Trp Thr Val Glu Asp Pro Val  
       610                   615                   620  
 Asp Ser Ala Pro Pro Gly Gln Arg Lys Lys Glu Gln Trp Tyr Ala Gly  
       625                   630                   635                   640  
 Ile Asn Pro Ser Asp Gly Ile Asn Ser Glu Val Leu Glu Ala Ile Arg  
       645                   650                   655  
 Val Thr Arg His Lys Asn Ala Met Ala Glu Arg Trp Glu Ser Arg Ile  
       660                   665                   670  
 Tyr Ala Ser Glu Glu Asp Asp  
       675

## (2) INFORMATION FOR SEQ ID NO:43:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: None

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Met Ser Pro Arg His Leu Ser Glu Ser Ser Gly Lys Pro Leu Ser Thr  
   1                   5                   10                   15  
 Lys Gln Glu Ala Ser Lys Pro Pro Arg Gly Cys Pro Gln Ala Asn Arg  
   20                   25                   30  
 Gly Val Val Arg  
   35

## (2) INFORMATION FOR SEQ ID NO:44:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: None

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Arg Leu Gln Lys Ser Gln Ser Ser Asp Leu Leu Glu Arg Glu Arg Glu  
   1                   5                   10                   15  
 Ser Val Leu Arg Arg Glu Gln Glu Val Ala Glu Glu Arg Arg Asn  
   20                   25                   30

## (2) INFORMATION FOR SEQ ID NO:45:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: None

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

-103-

- Ala Leu Phe Pro Glu Val Phe Ser Pro Thr Pro Asp Glu Asn Ser Asp  
 1 5 10 15  
 Gln Asn Ser Arg Ser Ser Ser Gln Ala Ser Gly Ile Thr Gly Ser Tyr  
 20 25 30  
 Ser Val Ser  
 35

## (2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Glu Asp Ser Val Asp Ser Ala Pro Pro Gly Gln Arg Lys Lys Glu Gln  
 1 5 10 15  
 Trp Tyr Ala Gly Ile Asn Pro Ser Asp Gly Ile Asn Ser Glu Val Leu  
 20 25 30  
 Glu

## (2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 215 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Met Ser Pro Arg His Leu Ser Glu Ser Ser Gly Lys Pro Leu Ser Thr  
 1 5 10 15  
 Lys Gln Glu Ala Ser Lys Pro Pro Arg Gly Cys Pro Gln Ala Asn Arg  
 20 25 30  
 Gly Val Val Arg Trp Glu Tyr Phe Arg Leu Arg Pro Leu Arg Phe Arg  
 35 40 45  
 Ala Pro Asp Glu Pro Gln Gln Ala Gln Val Pro His Val Trp Gly Trp  
 50 55 60  
 Glu Val Ala Gly Ala Pro Ala Leu Arg Leu Gln Lys Ser Gln Ser Ser  
 65 70 75 80  
 Asp Leu Leu Glu Arg Glu Arg Ser Val Leu Arg Arg Glu Gln Glu  
 85 90 95  
 Val Ala Glu Glu Arg Arg Asn Ala Leu Phe Pro Glu Val Phe Ser Pro  
 100 105 110  
 Thr Pro Asp Glu Asn Ser Asp Gln Asn Ser Arg Ser Ser Ser Gln Ala  
 115 120 125  
 Ser Gly Ile Thr Gly Ser Tyr Ser Val Ser Glu Ser Pro Phe Phe Ser  
 130 135 140  
 Pro Ile His Leu His Ser Asn Val Ala Trp Thr Val Glu Asp Pro Val  
 145 150 155 160  
 Asp Ser Ala Pro Pro Gly Gln Arg Lys Lys Glu Gln Trp Tyr Ala Gly  
 165 170 175  
 Ile Asn Pro Ser Asp Gly Ile Asn Ser Glu Val Leu Glu Ala Ile Arg  
 180 185 190  
 Val Thr Arg His Lys Asn Ala Met Ala Glu Arg Trp Glu Ser Arg Ile  
 195 200 205  
 Tyr Ala Ser Glu Glu Asp Asp  
 210 215

-104-

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Asp Tyr Lys Asp Asp Asp Lys  
1 5

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Met His Thr Glu His  
1 5 10 15  
His His His His His  
20

We Claim:

1. A method of detecting the presence of a target CS198 polynucleotide in a test sample, comprising:
  - (a) contacting said test sample with at least one CS198-specific polynucleotide or complement thereof; and
  - (b) detecting the presence of said target CS198 polynucleotide in the test sample, wherein said CS198-specific polynucleotide has at least 50% identity to a polynucleotide selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof.
2. The method of claim 1, wherein said target CS198 polynucleotide is attached to a solid phase prior to performing step (a).  
15
3. A method for detecting mRNA of CS198 in a test sample, comprising:
  - (a) performing reverse transcription with at least one primer in order to produce cDNA;
  - (b) amplifying the cDNA obtained from step (a) using CS198 oligonucleotides as sense and antisense primers to obtain CS198 amplicon; and
  - (c) detecting the presence of said CS198 amplicon in the test sample, wherein the CS198 oligonucleotides utilized in steps (a) and (b) have at least 50% identity to a polynucleotide selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof.  
20
4. The method of claim 3, wherein said test sample is reacted with a solid phase prior to performing one of steps (a), (b), or (c).  
25
5. The method of claim 3, wherein said detection step comprises utilizing a detectable label capable of generating a measurable signal.  
30
6. A method of detecting a target CS198 polynucleotide in a test sample suspected of containing said target, comprising:

- (a) contacting said test sample with at least one CS198 oligonucleotide as a sense primer and with at least one CS198 oligonucleotide as an anti-sense primer and amplifying to obtain a first stage reaction product;
- (b) contacting said first stage reaction product with at least one other 5 CS198 oligonucleotide to obtain a second stage reaction product, with the proviso that the other CS198 oligonucleotide is located 3' to the CS198 oligonucleotides utilized in step (a) and is complementary to said first stage reaction product; and
- (c) detecting said second stage reaction product as an indication of the presence of the target CS198 polynucleotide, wherein the CS198 oligonucleotides 10 utilized in steps (a) and (b) have at least 50% identity to a polynucleotide selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof.

7. The method of claim 6, wherein said test sample is reacted with a 15 solid phase prior to performing one of steps (a), (b), or (c).

8. The method of claim 6, wherein said detection step comprises utilizing a detectable label capable of generating a measurable signal.

20 9. The method of claim 8, wherein said detectable label is reacted to a solid phase.

10. A test kit useful for detecting CS198 polynucleotide in a test sample, comprising a container containing at least one CS198 polynucleotide having at least 25 50% identity with a sequence selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof.

11. A purified polynucleotide or fragment thereof derived from a CS198 gene, wherein said polynucleotide is capable of selectively hybridizing to the nucleic 30 acid of said CS198 gene and has at least 50% identity with a polynucleotide selected from the group consisting of (a) SEQUENCE ID NOS 7-13 and complements thereof, (b) SEQUENCE ID NOS 15-26 and complements thereof, and (c) fragments of SEQUENCE ID NOS 7-26.

35 12. The purified polynucleotide of claim 11, wherein said polynucleotide is produced by recombinant techniques.

13. The purified polynucleotide of claim 11, wherein said polynucleotide is produced by synthetic techniques.

5 14. The purified polynucleotide of claim 11, wherein said polynucleotide comprises a sequence encoding at least one CS198 epitope.

10 15. A recombinant expression system comprising a nucleic acid sequence that includes an open reading frame derived from CS198 operably linked to a control sequence compatible with a desired host, wherein said nucleic acid sequence has at least 50% identity to a polynucleotide selected from the group consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof.

15 16. A cell transfected with the recombinant expression system of claim 15.

20 17. A CS198 polypeptide having at least 50% identity with an amino acid sequence selected from the group consisting of (a) SEQUENCE ID NOS 43-47, and (b) fragments of SEQUENCE ID NOS 42-47

18. The polypeptide of claim 17, wherein said polypeptide is produced by recombinant techniques.

25 19. The polypeptide of claim 17, wherein said polypeptide is produced by synthetic techniques.

30 20. An antibody which specifically binds to at least one CS198 epitope, wherein said CS198 epitope is derived from an amino acid sequence having at least 50% identity with an amino acid sequence selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof.

35 21. An assay kit for determining the presence of CS198 antigen or anti-CS198 antibody in a test sample, comprising a container containing a CS198 polypeptide having at least 50% identity with an amino acid sequence selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof.

-108-

22. The assay kit of claim 21, wherein said polypeptide is attached to a solid phase.

23. An assay kit for determining the presence of CS198 antigen in a test sample, comprising a container containing an antibody which specifically binds to a CS198 antigen which comprises at least one CS198 epitope.

24. The kit of claim 23, wherein said antibody is attached to a solid phase.

10

25. A method for producing a polypeptide comprising at least one CS198 epitope, said method comprising incubating host cells that have been transfected with an expression vector containing a polynucleotide sequence encoding a polypeptide, wherein said polypeptide comprises an amino acid sequence having at least 50% identity with an amino acid sequence selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof.

26. A method for detecting CS198 antigen in a test sample suspected of containing said CS198 antigen, comprising:

20 (a) contacting the test sample with an antibody or fragment thereof which specifically binds to at least one epitope of a CS198 antigen selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof, wherein said contacting is carried out for a time and under conditions sufficient for the formation of antibody/antigen complexes; and

25 (b) detecting said complexes.

27. The method of claim 26, wherein said antibody is attached to a solid phase.

30 28. A method for detecting the presence of antibodies specific for a CS198 antigen in a test sample suspected of containing such antibodies, said method comprising:

35 (a) contacting the test sample with a CS198 polypeptide, wherein said CS198 polypeptide contains at least one CS198 epitope derived from an amino acid sequence or fragment thereof having at least 50% identity with an amino acid sequence selected from the group consisting of SEQUENCE ID NOS 42-47, and

-109-

fragments thereof, and further wherein said contacting is carried out for a time and under conditions sufficient to allow antigen/antibody complexes to form; and

(b) detecting said complexes.

5 29. The method of claim 28, wherein said CS198 polypeptide is attached to a solid phase.

30. A cell transfected with a nucleic acid sequence encoding at least one CS198 epitope, wherein said nucleic acid sequence is selected from the group 10 consisting of SEQUENCE ID NOS 1-27, and fragments or complements thereof.

31. A method for producing antibodies which specifically bind to CS198 antigen, comprising administering to an individual an isolated immunogenic polypeptide or fragment thereof in an amount sufficient to elicit an immune 15 response, wherein said immunogenic polypeptide comprises at least one CS198 epitope and has at least 50% identity with an amino acid sequence selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof.

32. A method for producing antibodies which specifically bind to CS198 20 antigen, comprising administering to an individual a plasmid comprising a sequence which encodes at least one CS198 epitope derived from a polypeptide having an amino acid sequence selected from the group consisting of SEQUENCE ID NOS 42-47, and fragments thereof.

25 33. A composition of matter comprising a CS198 polynucleotide or fragment thereof, wherein said polynucleotide has at least 50% identity with a polynucleotide selected from the group consisting of (a) SEQUENCE ID NOS 7-13 and complements thereof, (b) SEQUENCE ID NOS 15-26 and complements thereof, and (c) fragments of SEQUENCE ID NOS 7-26.

30 34. A composition of matter comprising a polypeptide containing at least one CS198 epitope, wherein said polypeptide has at least 50% identity with a sequence selected from the group consisting of (a) SEQUENCE ID NOS 43-47, and (b) fragments of SEQUENCE ID NOS 42-47.

35

-110-

35. The test kit of claim 10 further comprising a container with tools useful for collection of said sample, wherein the tools are selected from the group consisting of lancets, absorbent paper, cloth, swabs and cups.

5 36. The assay kit of claim 21 further comprising a container with tools useful for collection of said sample, wherein the tools are selected from the group consisting of lancets, absorbent paper, cloth, swabs and cups.

10 37. The test kit of claim 23 further comprising a container with tools useful for collection of said sample, wherein the tools are selected from the group consisting of lancets, absorbent paper, cloth, swabs and cups.

15 38. A gene, or a fragment thereof, which codes for a CS198 protein which comprises an amino acid sequence having at least 50% identity with SEQUENCE ID NO 47.

39. A gene, or a fragment thereof, comprising DNA having at least 50% identity with SEQUENCE ID NO 26.

1/12

>g2804590	CCTGCCTGCA CCCGCT: CAC CCTGAGCGCC TTGGGGTGGT GGGAGGCCT
>g2804590	GGAGGC TGCAGACTGT
>2682428	CCTGCCTGCA CCCGCT: CAC CCTGANCGCC TTGGGGTGGT GGGAGGCCT
>2682469	CCTGCCTGCA CCCGCTTCAC CCTGAGCGCC TTGGGGTGGT GGGANGCCT
>3359342	GGAGGC TGCAGACTGT
>1736231	TGCAGACTGT
>1734520	TGCAGACTGT
Consensus	CCTGCCTGCA CCCGCT: CAC CCTGAGCGCC TTGGGGTGGT TGCAGACTGT
>g2804590	GGAATCCCCA CTGTGCAG
>g2804590	GGAGCCGGGA GCCGGCAG
>g2804590	AGTA AGCCCAGAGG TCTCCACCCC A:CGGGAGGA
>2682428	GGAATCCCCA CTGTGCAGTA AGCCCAGATG TCTCCANNCC AACGGTAGGA
>2682469	GGAATCCCCA CTGTGCAGTA AGCCCAGAGG TCTCCACCCC A:CGGGATGA
>3359342	GGAGCCGGGA GCCGGCAGTA AGCCCAGAGG TCTCCACCCC A:CGGGAGGA
>1736231	GGAGCCGGGA GCCGGCAGTA AGCCCAGAGG TCTCCACCCC A:CGGGAGGA
>1734520	GGAGCCGGGA GCCGGCAGTA AGCCCAGAGG TCTCCACCCC A:CGGGAGGA
Consensus	GGAGCCGGGA GCCGGCAGTA AGCCCAGAGG TCTCCACCCC A:CGGGAGGA
>g2804590	AGGCTGAGGC CAAGACCCCG GAAGAGATGG ACCGCGTGAC CAGATAACCC
>2682428	AGGCTGATGC CAAGACCCCG GAAGAGATGG ANCGCGTGAC CAGATAACCC
>2682469	AGGCTGAGGC CAAGACCCCG GAAGAGATGG ACCGCGTGAC CAGATAACCC
>3359342	AGGCTGAGGC CAAGACCCCG GAAGAGATGG ACCGCGTGAC CAGATAACCC
>1736231	AGGCTGAGGC CAAGACCCCG GAAGAGATGG ACCGCGTGAC CAGATAACCC
>1734520	AGGCTGAGGC CAAGACCCCG GAAGAGATGG ACCGCGTGAC CAGATAACCC
>2596108	CCCCG GAAGAGATGG ACCGCGTGAC CAGATAACCC
Consensus	AGGCTGAGGC CAAGACCCCG GAAGAGATGG ACCGCGTGAC CAGATAACCC
>g2804590	ATCCTGGGCA TCCCTCAGGC ACACCGTGG: CACCGGCCTG GTGCTGGATG
>2682428	ATCCTNGCA TCCCTCAGGC ACACCGTGG: CACCGGCCTG GTGCTGGATG
>2682469	ATCCTGGGCA TCCCTCAGGC ACACCGTGG: CACCGGCCTG GTGCTNGATG
>3359342	ATCCTGGGCA TCCCTCAGGC ACACCGTGG: CACCGGT
>1736231	ATCCTGGGC
>1734520	ATCCTGGGCA TCCCTCAGGC ACACCGTGG: CACCGGCCTG GTGCTGGATG
>2596108	ATCCTGGGCA TCCCTCAGGC ACACCGTGG: CACCGGCCTG GTGCTGGATG
Consensus	ATCCTGGGCA TCCCTCAGGC ACACCGTGG: CACCGGCCTG GTGCTGGATG
>g2804590	GAGACACCAG CTACACATAC CATCTGGTGT GCATGGGCC CGAGGCCAGC
>2682428	GAGACACCAG CTACACATAC CATCTGGTGT GCATGGGCC CGAAGGCCAGC
>2682469	GAGACACNAG TTNCACATAC CATCTGGTGT GCATGGGCC CGANGC:AGC
>1734520	GAGACACCAG CTACACATAC CATCTGGTGT GCATGGGCC CGAGGCCAGC
>2596108	GAGACACCAG CTACACATAC CATCTGGTGT GCATGGGCC CGAGGCCAGC
>3388863	TAGGGCCAGC
Consensus	GAGACACCAG CTACACATAC CATCTGGTGT GCATGGGCC CGAGGCCAGC
>g2804590	GGCTGGGCC AGGATGAGCC GCAGACATGG CCCACTGACC ACAGGGCCA
>2682428	GGCTGGGCC ANGATGAGCC GCA:ACATGG CC:ACT
>2682469	GG: TGGGGC: AGGATGAGCC GCAGACATGG CC:ACTGACC NAAGGCCAT
>1734520	GG: TGGGGC: AGGATGAGCC
>2596108	GGCTGGGCC AGGATGAGCC GCAGACATGG CCCACTGACC ACAGGGCCA
>3388863	GGCTGGGCC AGGATGAGCC GCAGACATGG CCCACTGACC ACAGGGCCA
Consensus	GGCTGGGCC AGGATGAGCC GCAGACATGG CCCACTGACC ACAGGGCCA

FIG.1A

SUBSTITUTE SHEET (RULE 26)

2/12

>g2804590	GCAGGGCGTG	CAGAGGCAGG	GGGTGTCTA	CAGCGTGCAT	GCCTACACTG
>2596108	GCAGGGCGTG	CAGAGGCAGG	GGGTGTCTA	CAGCGTGCAT	GCCTACACTG
>3388863	GCAGGGCGTG	CAGAGGCAGG	GGGTGTCTA	CAGCGTGCAT	GCCTACACTG
Consensus	GCAGGGCGTG	CAGAGGCAGG	GGGTGTCTA	CAGCGTGCAT	GCCTACACTG
>g2804590	GCCAGCCGTC	CCCACGGGGG	CTCCACTCGG	AGAACAGGGG	GGATGAGGGT
>2596108	GCCAGCCGTC	CCCACGGGGG	CTCCACTCGG	AGAACAGGGG	GGATGAGGGT
>3388863	GCCAGCCGTC	CCCACGGGGG	CTCCACTCGG	AGAACAGGGG	GGATGAGGGT
Consensus	GCCAGCCGTC	CCCACGGGGG	CTCCACTCGG	AGAACAGGGG	GGATGAGGGT
>g2804590	TGGCAGGTTT	ACCGCCTGGG	CGCCAGGGAT	GCCCACCAGG	GACGTCCAAC
>3388863	TGGCAGGTTT	ACCGCCTGGG	CGCCAGGGAT	GCCCACCAGG	GACGTCCAAC
Consensus	TGGCAGGTTT	ACCGCCTGGG	CGCCAGGGAT	GCCCACCAGG	GACGTCCAAC
>g2804590	ATGGGCACTC	CGCCCAGAGG	ACGGGGAGGA	CAAGGAGATG	AAGACCTACC
>3388863	ATGGGCACTC	CGCCCAGAGG	ACGGGGAGGA	CAAGGAGATG	AAGACCTACC
<g2322685		CGCCCAGAGG	ACGGGGAGGA	CAAGGAGATG	AAGACCTACC
Consensus	ATGGGCACTC	CGCCCAGAGG	ACGGGGAGGA	CAAGGAGATG	AAGACCTACC
>g2804590	GCCTGGATGC	TGGGGACGCT	GACCCCAGGA	GGCTGTGTGA	CCTGGAGCGG
>3388863	GCCTGGATGC	TGGGGACGCT	GACCCCAGGA	GGCTGTGTGA	C: TGGAG
<g2322685	GCCTGGATGC	TGGGGACGCT	GACCCCAGGA	GGCTGTGTGA	CCTGGAGCGG
Consensus	GCCTGGATGC	TGGGGACGCT	GACCCCAGGA	GGCTGTGTGA	CCTGGAGCGG
>g2804590	GAGCGCTGGG	CCGTATCCA	GGGCCAGGCA	GTCAGGAAGA	GCAGCACCGT
<g2322685	GAGCGCTGGG	CCGTATCCA	GGGCCAGGCA	GTCAGGAAGA	GCAGCACCGT
Consensus	GAGCGCTGGG	CCGTATCCA	GGGCCAGGCA	GTCAGGAAGA	GCAGCACCGT
>g2804590	GGCCACGCTC	CAGGGCACTC	CTGACCACGG	AGACCCCAGG	ACCCCCGGCC
<g2322685	GGCCACGCTC	CAGGGCACTC	CTGACCACGG	AGACCCCAGG	ACCCCCGGCC
Consensus	GGCCACGCTC	CAGGGCACTC	CTGACCACGG	AGACCCCAGG	ACCCCCGGCC
>g2804590	CACCTCGGTC	CACGCCCTG	GAGGAGAACG	TGGTTGACAG	GGAGCAGATT
<g2322685	CACCTCGGTC	CACGCCCTG	GAGGAGAACG	TGGTTGACAG	GGAGCAGATT
Consensus	CACCTCGGTC	CACGCCCTG	GAGGAGAACG	TGGTTGACAG	GGAGCAGATT
>g2804590	GACTTCCTGG	CAGCGAGACA	GCAGTTCTG	AGTCTGGAGC	AGGCGAACAA
<g2322685	GACTTCCTGG	CAGCGAGACA	GCAGTTCTG	AGTCTGGAGC	AGGCGAACAA
>3988413				GGAGC	AGGCGAACAA
Consensus	GACTTCCTGG	CAGCGAGACA	GCAGTTCTG	AGTCTGGAGC	AGGCGAACAA
>g2804590	GGGGGCCCCCT	CATAGCTCCC	CGGCCAGGGG	GACCCCTGCA	GGCACAAACCC
>3988413	GGGGGCCCCCT	CATAGCTCCC	CGGCCAGGGG	GACCCCTGCA	GGCACAAACCC
Consensus	GGGGGCCCCCT	CATAGCTCCC	CGGCCAGGGG	GACCCCTGCA	GGCACAAACCC
>g2804590	CAGGGGCCAG	CCAGGGCCCC	AAGGCCTTCA	ACAAGCCCCA	CCTGGCCAAC
>3988413	CAGGGGCCAG	CCAGGGCCCC	AAGGCCTTCA	ACAAGCCCCA	CCTGGCCAAC
Consensus	CAGGGGCCAG	CCAGGGCCCC	AAGGCCTTCA	ACAAGCCCCA	CCTGGCCAAC
>g2804590	GGGCACGTGG	TTCCCATCAA	GCCCCAGGTG	AAGGGGGTGG	TCAGGGAAGA
>3988413	GGGCACGTGG	TTCCCATCAA	GCCCCAGGTG	AAGGGGGTGG	TCAGGGAAGA
Consensus	GGGCACGTGG	TTCCCATCAA	GCCCCAGGTG	AAGGGGGTGG	TCAGGGAAGA

FIG.1B

SUBSTITUTE SHEET (RULE 26)

3/12

>g2804590	GAACAAGGTG	CGTGCTGTGC	CCACCTGGGC	CAGTGTCAA	GTTGTGGATG
>3988413	GAACAAGGTG	CGTGCTGTGC	CCACCTGGGC	CAGTGTCAA	GTTGTGGATG
Consensus	GAACAAGGTG	CGTGCTGTGC	CCACCTGGGC	CAGTGTCAA	GTTGTGGATG
>g2804590	ACCCCTGGCTC	CTTGGCCTCA	GTGGAGTCCC	CGGGGACCCC	CAAGGAGACG
>3988413	ACCCCTGGCTC	CTTGGCCTCA	GTGGAGTCCC	CGGGGACCCC	CAAGGAGACG
Consensus	ACCCCTGGCTC	CTTGGCCTCA	GTGGAGTCCC	CGGGGACCCC	CAAGGAGACG
>g2804590	CCCATCGAGC	GGGAGATCCG	TCTGGCTCAG	GAGCGTGAGG	CAGACCTGCG
>3988413	CCCATCGAGC	GGGAGATCCG	TCTGGCTCAG		
Consensus	CCCATCGAGC	GGGAGATCCG	TCTGGCTCAG	GAGCGTGAGG	CAGACCTGCG
>g2804590	AGAGCAGAGG	GGGCTTCGGC	AGGCAACCGA	CCACCAGGAG	CTGGTGGAAA
Consensus	AGAGCAGAGG	GGGCTTCGGC	AGGCAACCGA	CCACCAGGAG	CTGGTGGAAA
>g2804590	TCCCCACCAAG	GCCGCTGCTG	ACCAAGCTGA	GCCTGATCAC	AGCCCCACGG
Consensus	TCCCCACCAAG	GCCGCTGCTG	ACCAAGCTGA	GCCTGATCAC	AGCCCCACGG
>g2804590	CGGGAGAGAG	GGCGCCCGTC	CCTCTACGTG	CAGCGG:GAC	ATAGTACAGG
>3615515				TG	CAGCGGNGAC
Consensus	CGGGAGAGAG	GGCGCCCGTC	CCTCTACGTG	CAGCGGNGAC	ATAGTACAGG
>g2804590	AGACACAGCG	TGAGGAAGAC	CACCGGCAGGG	AGGGCCTGCA	CGTGGGCCGG
>3615515	AGACACAGCG	TGAGGAAGAC	CACCGGCAGGG	AGGGCCTGCA	CGTGGGCCGG
Consensus	AGACACAGCG	TGAGGAAGAC	CACCGGCAGGG	AGGGCCTGCA	CGTGGGCCGG
>g2804590	GCGTCCACAC	CCGACTGGGT	CTCGGAGGGT	CCCCAGCCCG	GACTCCGGAG
>3615515	GCGTCCACAC	CCGACTGGGT	CTCGGAGGGT	CCCCAGCCCG	GACTCCGGAG
Consensus	GCGTCCACAC	CCGACTGGGT	CTCGGAGGGT	CCCCAGCCCG	GACTCCGGAG
>g2804590	AGCCCTCAGC	TCAGATTCCA	TCCTCAGCCC	GGCCCCAGAT	GCCC GTGCGG
>3615515	AGCCCTCAGC	TCAGATTCCA	TCCTCAGCCC	GGCCCCAGAT	GCCC GTGCGG
Consensus	AGCCCTCAGC	TCAGATTCCA	TCCTCAGCCC	GGCCCCAGAT	GCCC GTGCGG
>g2804590	CCGACCCAGC	TCCAGAAAGTG	AGGAAGGTGA	ACCGCATCCC	ACCTGATGCC
>3615515	CCGACCCAGC	TCCAGAAAGTG	AGGAAGGTGA	ACCGCATCCC	ACCTGATGCC
>2055371					CTGATGCC
>2055371IH					TGATGCC
Consensus	CCGACCCAGC	TCCAGAAAGTG	AGGAAGGTGA	ACCGCATCCC	ACCTGATGCC
>g2804590	TACCAGCCGT	ACCTGAGCCC	CGGGACCCCC	CAGCTAGAAT	TCTCAGCCCT
>3615515	TACCAGCCGT	ACCTGAGCCC	CGGGACCCCC	CAGCTAGNAT	TCTCAGC:TT
>2055371	TACCAGCCGT	ACCTGAGCCC	CGGGACCCCC	CAGCTAGAAT	TCTCAGCCCT
>2055371IH	TACCAGCCGT	ACCTGAGCCC	CGGGACCCCC	CAGCTAGAAT	TCTCAGCCCT
Consensus	TACCAGCCGT	ACCTGAGCCC	CGGGACCCCC	CAGCTAGAAT	TCTCAGCCCT
>g2804590	CGGAGCATT	GGCAAGCCCC	GCAGTCTCTC	CACAGCGGAG	G:CCAAGGCT
>3615515	CGGAGCATT	GGCAAGCCCC	G		
>2055371	CGGAGCATT	GGCAAGCCCC	GCAGTCTCTC	NACAGCGGAG	GANCAAGGCT
>2055371IH	CGGAGCATT	GGCAAGCCCC	GCAGTCTCTC	CACAGCGGAG	G:CCAAGGCT
>1431231			CTCTC	CACAGCGGAG	G:CCAAGGCT
Consensus	CGGAGCATT	GGCAAGCCCC	GCAGTCTCTC	CACAGCGGAG	G:CCAAGGCT

FIG.1C

4/12

>g2804590 GCGACTTCAC CAAAGGCCAC GATGTCCCCG AGGCATCTCT CAGAATCCTC  
 >2055371 GCGACTTCAC CAAAGGCCAC GATGTCCCCG AGGCATCTCT CAGAATCCTC  
 >2055371IH GCGACTTCAC CAAAGGCCAC GATGTCCCCG AGGCATCTCT CAGAATCCTC  
 >1431231 GCGACTTCAC CAAAGGCCAC GATGTCCCCG AGGCATCTCT CAGAATCCTC  
 Consensus GCGACTTCAC CAAAGGCCAC GATGTCCCCG AGGCATCTCT CAGAATCCTC  
  
 >g2804590 TGGAAAACCC CTGAGCACAA AGCAAGAGGC ATCGAAGCCC CCTCGGGGAT  
 >2055371 TGGAAAACCC CTGAGCACAA AGCAAGAGGC ATCGAAGCCC CCTCGGGGAT  
 >2055371IH TGGAAAACCC CTGAGCACAA AGCAAGAGGC ATCGAAGCCC CCTCGGGGAT  
 >1431231 TGGAAAACCC CTGAGCACAA AGCAAGAGGC ATCGAAGCCC CCTCGGGGAT  
 >3253860 CCCCCGCAAGC CAACAGGGGT GTCGTGCGGT GGGAGTACTT CCGCCTGCGT  
 >2055371 GCCCCGCAAGC CAACAGGGGT GTCGTGCGGT GGGAGTACTT CCGCCTGCGT  
 >2055371IH GCCCCGCAAGC CAACAGGGGT GTCGTGCGGT GGGAGTACTT CCGCCTGCGT  
 >1431231 GCCCCGCAAGC CAACAGGGGT GTCGTGCGGT GGGAGTACTT CCGCCTGCGT  
 >3253860 GCCCCGCAAGC CAACAGGGGT GTCGTGCGGT GGGAGTACTT CCGCCTGCGT  
 Consensus GCCCCGCAAGC CAACAGGGGT GTCGTGCGGT GGGAGTACTT CCGCCTGCGT  
  
 >g2804590 CCTCTGCGGT TCAGGGCCCC AGACGAGCCC CAGCAGGCC AAGTCCCCA  
 >2055371IH CCTCTGCGGT TCAGGGCCCC AGACGAGCCC CAGCAGGCC AAGTCCCCA  
 >1431231 CCTCTGCGGT TCAGGGCCCC AGACGAGCCC CA  
 >3253860 CCTCTGCGGT TCAGGGCCCC AGACGAWCCC CAGCAGGCC AAGTCCCCA  
 Consensus CCTCTGCGGT TCAGGGCCCC AGACGAGCCC CAGCAGGCC AAGTCCCCA  
  
 >g2804590 TGTCTGGGC TGGAAGGTGG CTGGGGCCCC TGCACTGAGG CTGCAGAAGT  
 >2055371IH TGTCTGGGC TGGAAGGTGG CTGGGGCCCC TGCACTGAGG CTGCAGAAGT  
 >3253860 TGTCTGGGC TGGAAGGTGG CTGGGGCCCC TGCACTGAGG CTGCAGAAGT  
 >1753756 GGAGGTGG CTGGGGCCCC NGCACTGAGG CTGCAGAAGT  
 Consensus TGTCTGGGC TGGAAGGTGG CTGGGGCCCC TGCACTGAGG CTGCAGAAGT  
  
 >g2804590 CCCAGTCATC TGATCTGCTG GAAAGGGAGA GGGAGAGTGT CCTGCGCCGG  
 >2055371IH CCCAGTCATC TGATCTGCTG GAAAGGGAGA GGGAGAGTGT CCTGCGCCGG  
 >3253860 CCCAGTCATC TGATCTGCTG GAAAGGGAGA GGGAGAGTGT CCTGCGCCGG  
 >1753756 CCCAGTCATC TGATCTGCTG GAAAGGGAGA GGGAGAGTGT CCTGCGCCGG  
 Consensus CCCAGTCATC TGATCTGCTG GAAAGGGAGA GGGAGAGTGT CCTGCGCCGG  
  
 >g2804590 GAGCAAGAGG TGGCAGAGGA GCGGAGAAAT GCTCTTCC CAGAGGTCTT  
 >2055371IH GAGCAAGAGG TGGCAGAGGA GCGGAGAAAT GCTCTTCC CAGAGGTCTT  
 >3253860 GAGCAAGAGG TGGCAGAGGA GCGGAGAAAT GCTCTTCC CAGAGGTCTT  
 >1753756 GAGCAAGAGG TGGCAGAGGA GCGGAGAAAT GCTCTTCC CAGAGGTCTT  
 Consensus GAGCAAGAGG TGGCAGAGGA GCGGAGAAAT GCTCTTCC CAGAGGTCTT  
  
 >g2804590 CTCCCCAACG CCAGATGAGA ACTCTGACCA GAACTCCAGG AGCTCCTCCC  
 >2055371IH CTCCCCAACG CCAGATGAGA ACTCTGACCA GAACTCCAGG AGCTCCTCCC  
 >3253860 C  
 >1753756 CTCCCCAACG CCAGATGAGA ACTCTGACCA GAACTCCAGG AGCTCCTCCC  
 Consensus CTCCCCAACG CCAGATGAGA ACTCTGACCA GAACTCCAGG AGCTCCTCCC

FIG.1D

SUBSTITUTE SHEET (RULE 26)

5/12

>g2804590 AGGCATCCGG  
 >g2804590 GG CATCACGGGC AGTTACTCGG TGTCTGAGTC TCCCTTCTTC  
 >2055371IH AGGCATCCGG CATCACGGGC AGTTACTCGG TGTCTGAGTC TCCCTTCTTC  
 >1753756 AGGCATCCGG CATCACGGGC AGTTA:TCGG TGTCTGAGTC TCCCTTCTTC  
 >1887713 C TCCCTTCTTC  
 Consensus AGGCATCCGG CATCACGGGC AGTTACTCGG TGTCTGAGTC TCCCTTCTTC  
  
 >g2804590 AGCCCCATCC ACCTACACTC AAACGTGGCG TGGACAGTGG AAGATCCAGT  
 >2055371IH AGCCCCATCC ACCTACACTC AAACGTGGCG TGGACAGTGG AAGATCCAGT  
 >1887713 AGCCCCATCC ACCTACACTC AAACGTGGCG TGGACAGTGG AAGATCCAGT  
 Consensus AGCCCCATCC ACCTACACTC AAACGTGGCG TGGACAGTGG AAGATCCAGT  
  
 >g2804590 GGACAGTGCT CCTCCCGGGC AGAGAAAGAA GGAGCAATGG G TACGCTGGCA  
 >g2804590 GGACAGTGCT CCTCCCGGGC AGAGAAAGAA GGAGCAATGG TACGCTGGCA  
 >2055371IH GGACAGTGCT CCTCCCGGGC AGAGAAAGAA GGAGCAATGG TACGCTGGCA  
 >1887713 GGACAGTGCT CCTCCCGGGC AGAGAAAGAA GGAGCAATGG TACGCTGGCA  
 Consensus GGACAGTGCT CCTCCCGGGC AGAGAAAGAA GGAGCAATGG TACGCTGGCA  
  
 >g2804590 TCAACCCCTC GGACGGTATC AACTCAGAGG T  
 >g2804590 AGG TCCTGGAAGC CATACTGGTG  
 >2055371IH TCAACCCCTC GGACGGTATC AACTCAGAGG TCCTGGAAGC CATACTGGTG  
 >1887713 TCAACCCCTC GGACGGTATC AACTCAGAGG TCCTGGAAGC CATACTGGTG  
 Consensus TCAACCCCTC GGACGGTATC AACTCAGAGG TCCTGGAAGC CATACTGGTG  
  
 >g2804590 ACCCGTCACA AGAACGCCAT GGCAGAGCGC TGGGAATCCC GCATCTACGC  
 >2055371IH ACCCGTCACA AGAACGCCAT GGCAGAGCGC TGGGAATCCC GCATCTACGC  
 >1887713 ACCCGTCACA AGAACGCCAT GGCAGAGCGC TGGGAATCCC GCATCTACGC  
 >1803052 C TGGGAATCCC GCATCTACGC  
 Consensus ACCCGTCACA AGAACGCCAT GGCAGAGCGC TGGGAATCCC GCATCTACGC  
  
 >g2804590 CAGTGAGGAG GATGACTGAG CCTCGGGATG GGGCGCCAC CCCCTGCCCT  
 >2055371IH CAGTGAGGAG GATGACTGAG CCTCGGGATG GGGCGCCAC CCCCTGCCCT  
 >1887713 CAGTGAGGAG GATGACTGAG CCTCGGGATG GGGCGCCAC CCCCTGCCCT  
 >1803052 CAGTGAGGAG GATGACTGAG CCTCGGGATG GGGCGCCAC CCCCTGCCCT  
 Consensus CAGTGAGGAG GATGACTGAG CCTCGGGATG GGGCGCCAC CCCCTGCCCT  
  
 >g2804590 GCCCTGACCC TCGTGGGAAC TGCCAAGACC ATCGCCAAGC CCCCCACCTA  
 >2055371IH GCCCTGACCC TCGTGGGAAC TGCCAAGACC ATCGCCAAGC CCCCCACCTA  
 >1887713 GCCCTGACCC TCGTGGGAAC TGCCAAGACC ATCGCCAAGC CCCCCACCTA  
 >1803052 GCCCTGACCC TCGTGGGAAC TGCCAAGACC ATCGCCAAGC CCCCCACCTA  
 Consensus GCCCTGACCC TCGTGGGAAC TGCCAAGACC ATCGCCAAGC CCCCCACCTA  
  
 >g2804590 GGAAATGGGT CCTAGGTCCA GGATCCAAGA ACCACAGCTC ATCTGCCAAC  
 >2055371IH GGAAATGGGT CCTAGGTCCA GGATCCAAGA ACCACAGCTC ATCTGCCAAC  
 >1803052 GGAAATGGGT CCTAGGTCCA GGATCCAAGA ACCACAGCTC ATCTGCCAAC  
 Consensus GGAAATGGGT CCTAGGTCCA GGATCCAAGA ACCACAGCTC ATCTGCCAAC  
  
 >g2804590 AATCCACCA TGGGCACATT TGGGACTGTT GGGTTTTTCG TTTCCGTTTC  
 >2055371IH AATCCACCA TGGGCACATT TGGGACTGTT GGGTTTTTCG TTTCCGTTTC  
 >1803052 AATCCACCA TGGGCACATT TGGGACTGTT GGGTTTTTCG TTTCCGTTTC  
 Consensus AATCCACCA TGGGCACATT TGGGACTGTT GGGTTTTTCG TTTCCGTTTC

FIG. 1E  
SUBSTITUTE SHEET (RULE 26)

6/12

>g2804590 TATCTTCCTT TAGAAATGTT TCTGCCTTTG GGGTCTAAAG CTTTTGGGA  
 >2055371IH TATCTTCCTT TAGAAATGTT TCTGCCTTTG GGGTCTAAAG CTTTTGGGA  
 >1803052 TATCTTCCTT TAGAAATGTT TCTGCCTTTG GGGTCTAAAG CTTTTGGGA  
 >889029 G CTTTTGGGA  
 Consensus TATCTTCCTT TAGAAATGTT TCTGCCTTTG GGGTCTAAAG CTTTTGGGA  
  
 >g2804590 TGAAATGGGA CCCCTGCTGA TTCTTTCTGC TTCTAAGACT TTGCCAAATG  
 >2055371IH TGAAATGGGA CCCCTGCTGA TTCTTTCTGC TTCTAAGACT TTGCCAAATG  
 >1803052 TGAAATGGGA CCC  
 >889029 TGAAATGGGA CCCCTGCTGA TTCTTTCTGC TTCTAAGACT TTGCCAAATG  
 Consensus TGAAATGGGA CCCCTGCTGA TTCTTTCTGC TTCTAAGACT TTGCCAAATG  
  
 >g2804590 CCCTGGGTCT AAGAAAGAAA GAGACCCGCT CCTCCACTTT CAGGTGTAAT  
 >2055371IH CCCTGGGTCT AAGAAAGAAA GAGACCCGCT CCTCCACTTT CAGGTGTAAT  
 >889029 CCCTGGGTCT AAGAAAGAAA GAGACCCGCT CCTCCACTTT CAGGTGTAAT  
 >2620906 GAAAGAAA GAGACCCGCT CCTCCACTTT CAGGTGTAAT  
 Consensus CCCTGGGTCT AAGAAAGAAA GAGACCCGCT CCTCCACTTT CAGGTGTAAT  
  
 >g2804590 TTGCTTCCGC TAGTCTGAGG GCAGAGGGAC CGGTCAAAGA GGGTGGCACA  
 >2055371IH TTGCTTCCGC TAGTCTGAGG GCAGAGGGAC CGGTCAAAGA GGGTGGCACA  
 >889029 TTGCTTCCGC TAGTCTGAGG GCAGAGGGAC CGGTCAAAGA GGGTGGCACA  
 >2620906 TTGCTTCCGC TAGTCTGAGG GCAGAGGGAC CGGTCAAAGA GGGTGGCACA  
 Consensus TTGCTTCCGC TAGTCTGAGG GCAGAGGGAC CGGTCAAAGA GGGTGGCACA  
  
 >g2804590 GATCGCAGCA CCTTGAGGGG CTGCGGGTCT GAGGGAGGAG ACACTCAGCT  
 >2055371IH GATCGCAGCA CCTTGAGGGG CTGCGGGTCT GAGGGAGGAG ACACTCAGCT  
 >889029 GATCGCAGCA CCTTGAGGGG CTGCGGGTCT GAGGGAGGAG ACACTCAGCT  
 >2620906 GATCGCAGCA CCTTGAGGGG CTGCGGGTCT GAGGGAGGAG ACACTCAGCT  
 Consensus GATCGCAGCA CCTTGAGGGG CTGCGGGTCT GAGGGAGGAG ACACTCAGCT  
  
 >g2804590 CCTCCCTCTG AGAAGTCCA AGCTGAGAGG GGAGACCTGC CCCTTCCAA  
 >2055371IH CCTCCCTCTG AGAAGTCCA AGCTGAGAGG GGAGACCTGC CCCTTCCAA  
 >889029 CCTCCCTCTG AGAAGTCCA AGCTGAGAGG GGAGACCTGC CCCTTCCAA  
 >2620906 CCTCCCTCTG AGAAGTCCA AGCTGAGAGG GGAGACCTGC CCCTTCCAA  
 Consensus CCTCCCTCTG AGAAGTCCA AGCTGAGAGG GGAGACCTGC CCCTTCCAA  
  
 >g2804590 CCCTGGAAA CCATCCAGTC TGAGGGAGGA GGCCAAACTC CCAGTGCTGG  
 >2055371IH CCCTGGAAA CCATCCAGTC TGAGGGAGGA GGCCAAACTC CCAGTGCTGG  
 >889029 CCCTGGAAA CCATCCAGTC TGAGGGAGGA GGCCAAACTT CCAGTGCTGG  
 >2620906 CCCTGGAAA CCATCCAGTC TGAGGGAGGA GGCCAAACTC CCAGTGCTGG  
 Consensus CCCTGGAAA CCATCCAGTC TGAGGGAGGA GGCCAAACTC CCAGTGCTGG  
  
 >g2804590 GGGTCCCTGT GCAGCCCTCA AACCCCTCAC CTTGGTGCAC CCAGCCACAC  
 >2055371IH GGGTCCCTGT GCAGCCCTCA AACCCCTCAC CTTGGTGCAC CCAGCCACAC  
 >889029 GGGTCCCTGT GCA  
 >2620906 GGGTCCCTGT GCAGCCCTCA AACCCCTTC  
 >1754901 GGGTCCCTGT GCAGCCCTCA AACCCCTCAC CTTGGTGCAC CCAGCCACAC  
 Consensus GGGTCCCTGT GCAGCCCTCA AACCCCTCAC CTTGGTGCAC CCAGCCACAC  
  
 >g2804590 CTGGTGGACA CAAAGCTCTC ACATCGATAG GATCCCATGA GGATGGTCCC  
 >2055371IH CTGGTGGACA CAAAGCTCTC ACATCGATAG GATCCCATGA GGATGGTCCC  
 >1754901 CTGGTGGACA CAAAGCTCTC ACATCGATAG GATCCCATGA GGATGGTCCC  
 Consensus CTGGTGGACA CAAAGCTCTC ACATCGATAG GATCCCATGA GGATGGTCCC

**FIG.1F**  
 SUBSTITUTE SHEET (RULE 26)

7/12

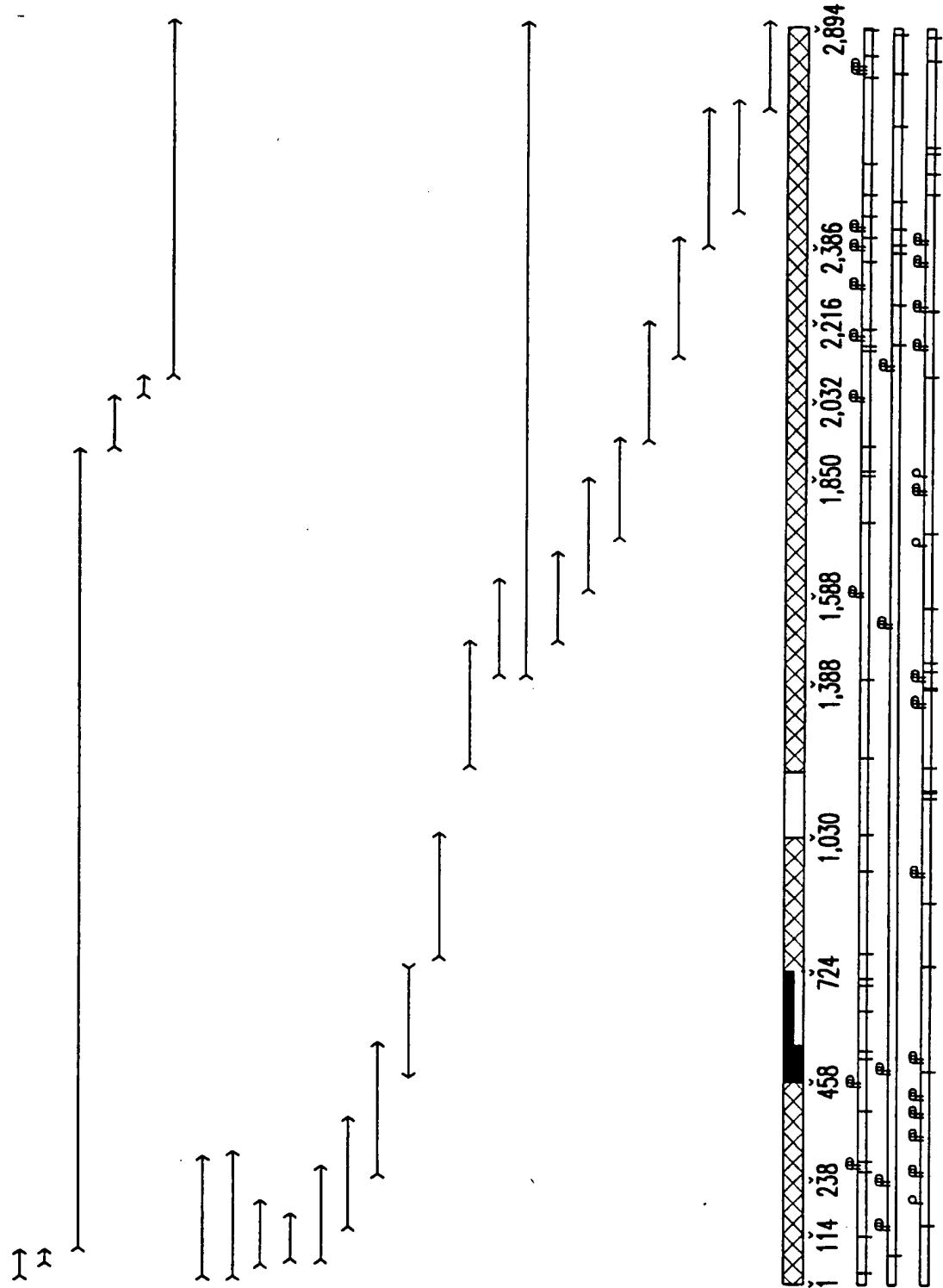
>g2804590 CTTCACCTGG GAGAAAAGTG ACCCAGTTA GGAGCTGGAG GGGGGTCTTT  
>2055371IH CTTCACCTGG GAGAAAAGTG ACCCAGTTA GGAGCTGGAG GGGGGTCTTT  
>1754901 CTTCACCTGG GAGAAAAGTG ACCCAGTTA GGAGCTGGAG GGGGGTCTTT  
Consensus CTTCACCTGG GAGAAAAGTG ACCCAGTTA GGAGCTGGAG GGGGGTCTTT

>g2804590 GTCCCCCACC CCCAAACTGC CCTGAAATAA ACCTGGAGTG AGCTGCC  
>2055371IH GTCCCCCACC CCCAAACTGC CCTGAAATAA ACCTGGAGTG AGCTGCCCA  
>1754901 GTCCCCCACC CCCAAACTGC CCTGAAATAA ACCTGGAGTG AGCTGCC  
Consensus GTCCCCCACC CCCAAACTGC CCTGAAATAA ACCTGGAGTG AGCTGCCCA

FIG. 1G

SUBSTITUTE SHEET (RULE 26)

8/12



92804590-1  
92804590-2  
92804590-3  
92804590-4  
92804590-5  
92804590-6

33888863 92322895  
3988413 3615515  
2055371 1H  
2055371  
1431231  
32538660  
1753756  
1887713  
1803052  
889029  
2620906  
1753901  
SUBSTITUTE SHEET (RULE 26)

694415GA1-1

FIG. 2

12

9 / 12

11

10

9

8

7

6

5

4

3

2

1

0

-1

-2

-3

-4

-5

-6

-7

-8

-9

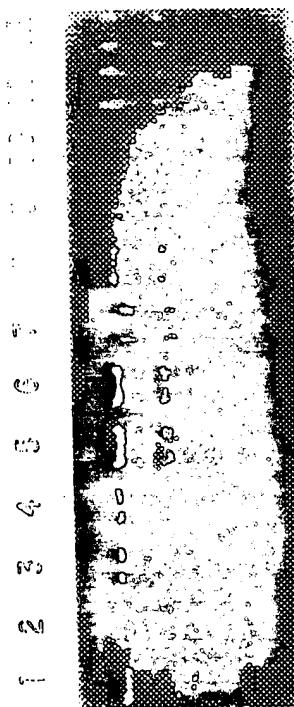
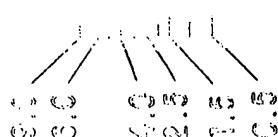
-10

-11

-12

Tissue  
 Bladder  
 Breast  
 Colon  
 Kidney  
 Liver  
 Lung  
 Muscle  
 Ovary  
 Placenta  
 Prostate  
 Spleen  
 Testis

1  
 2  
 3  
 4  
 5  
 6  
 7  
 8  
 9  
 10  
 11  
 12



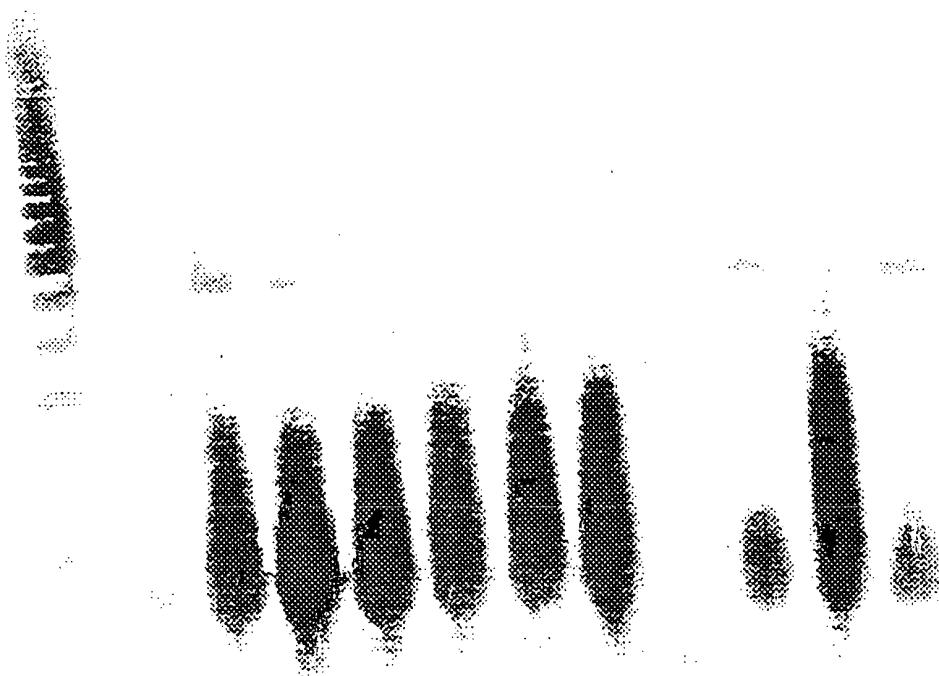
Time  
 1 2 3 4 5 6 7 8 9 10 11 12

1  
 2  
 3  
 4  
 5  
 6  
 7  
 8  
 9  
 10  
 11  
 12

SUBSTITUTE SHEET (RULE 26)

10/12

1 2 3 4 5 6 7 8 9 10 11 12



Line	Sample
1	DNA Molecular Weight Marker
2	Placental DNA control
3	normal colon
4	cancer colon
5	cancer breast
6	cancer breast
7	BPH prostate
8	cancer prostate
9	BPH prostate
10	normal lung
11	normal lung
12	cancer lung

PCT/US98/06251

SUBSTITUTE SHEET (RULE 26)

11/12



Lane	Contents
1	DNA Molecular Weight Marker (100 bp)
2	placental DNA control
3	normal colon
4	cancer colon
5	cancer colon
6	normal colon
7	cancer colon

FIG. 5

SUBSTITUTE SHEET (RULE 26)

12 / 12

1 2 3 4 5 6 7 8 9 10 11 12 13 14



Lane 1	Normal breast
Lane 2	Normal liver
Lane 3	Normal lung
Lane 4	ESL prostate
Lane 5	Ovarian cancer
Lane 6	Normal bladder
Lane 7	Bladder cancer
Lane 8	Normal colon
Lane 9	Normal colon
Lane 10	Normal colon
Lane 11	Colon cancer
Lane 12	Colon cancer
Lane 13	Colon cancer
Lane 14	Marker's

FIG. 6  
SUBSTITUTE SHEET (RULE 26)

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 98/06251

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 6 C12Q1/68 C07K14/47 C12N5/10 C07K16/00 G01N33/574  
A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C12N C12Q C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 39419 A (HUMAN GENOME SCIENCES INC ; YU GUO LIANG (US); ROSEN CRAIG A (US)) 12 December 1996	1-27, 30-39
Y	see the whole document	28,29
Y	---	28,29
X	WO 93 11435 A (COHEN STEVEN J) 10 June 1993 see esp. page 7 ff	1,10,11
X	EMBL database entry HSAA33671, AC#AA133671; 06. Dec. 1996, Hillier L. et al.,:stratagene colon, homo sapiens cDNA clone XP002073288 abstract	---
	---	-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

\* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority, claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*Z\* document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 August 1998	26.08.1998
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Müller, F.

## INTERNATIONAL SEARCH REPORT

Inte...nal Application No

PCT/US 98/06251

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL database entry HSAA60684, AC#AA160684; 19. Jan. 1997, Hillier et al., :Stratagene pancreas homo sapiens cDNA clone, XP002073289 abstract ---	1,10,11
X	EMBL database entry HSAA43765, AC#AA143765, 14. Dec. 1996; Hillier et al.: stratagene colon cDNA clone, XP002073290 abstract ---	1,10,11
A	WO 95 20681 A (INCYTE PHARMA INC) 3 August 1995 see the whole document ---	1-39
X,P	EMBL Database entry AC004030, AC004030; 26. Jan. 1998, Lamerdin et al.,: Homo sapiens DNA chromosome 19, XP002073284 abstract ---	1,10
P,X	EMBL database, AC# AA552295, 11. Sep. 1997, Homo sapiens cDNA clone XP002073293 abstract -----	1,10,11

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 98/06251

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9639419	A 12-12-1996	AU 2820595	A 24-12-1996	EP 0847398 A 17-06-1998
WO 9311435	A 10-06-1993	CA 2124319	A 10-06-1993	CA 2200363 A 26-05-1993
		EP 0643838	A 22-03-1995	JP 7509557 T 19-10-1995
		US 5637453	A 10-06-1997	ZA 9209160 A 16-08-1993
WO 9520681	A 03-08-1995	AU 688465	B 12-03-1998	AU 1694695 A 15-08-1995
		BG 100751	A 31-07-1997	BR 9506657 A 16-09-1997
		CA 2182217	A 03-08-1995	CN 1145098 A 12-03-1997
		CZ 9602189	A 14-05-1997	EP 0748390 A 18-12-1996
		FI 962987	A 26-09-1996	JP 9503921 T 22-04-1997
		LV 11696	B 20-08-1997	LV 11696 B 20-08-1997
		NO 963151	A 27-09-1996	PL 315687 A 25-11-1996
		HU 75550	A 28-05-1997	HU 75550 A 28-05-1997

THIS PAGE BLANK (USPTO)

